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Original research paper

INQUIRY FOR SUITABLE LOCATIONS FOR A DRILLING REGIME AT AN UPSLOPE ROCKY KNOLL OF LAWU ESTATE, WESTERN BYPASS, MINNA, NIGERIA

INVESTIGAÇÃO DE LOCAIS ADEQUADOS PARA UM REGIME DE PERFURAÇÃO EM UMA ELEVAÇÃO ROCHOSA NA ENCOSTA SUPERIOR DA PROPRIEDADE LAWU, CONTORNO OESTE, MINNA, NIGÉRIA

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ABSTRACT

Background: A client requested that the study group help determine locations that would be suitable for a drilling regime at his lot, located at an upslope rocky knoll of Lawu Estate, Minna, Nigeria. **Aim:** The aim of this study is to carry out a purpose-specific survey to pinpoint the best locations in a built-up property at the upmarket Lawu Estate that would be suitable for a drilling regime targeted for household consumption. **Methods:** The study area was reconnoitered by the survey crew in order to georeference the locations that would be occupied for the vertical electrical sounding survey in the 30 m x 20 m lot. Owing to the extensive build-up at this lot, only a four-point traverse along the 30-metric dimension traverse of the frontage of the building was demarcated in the northeasterly direction, thereby limiting the desire of the survey crew to define an appropriate survey grid. The data-acquisition pattern at the 4 x 1 survey stations of the frontage-traverse of the lot followed the “traditional” sequence of Schlumberger array layout measurements, whence the survey crew progressed with current-electrode spacing either end of a survey point located at this frontage-traverse targeting a maximum survey depth of 100 m. **Result:** The acquired vertical electrical-sounding data set for this study was recorded on purpose-specific data sheets. **Discussion:** Based on empirical rules-of-thumb procedures for interpreting vertical electrical sounding data at the Nigerian Basement Complex geological province, “assured” groundwater location and “strongly aquiferous” location, deductive inferences were drawn with regards to only vertical electrical sounding Station 4. **Conclusion:** Thus, it is recommended that VES Station 4 be exploited in the planned drilling program of the client, especially since this survey point checks off 100 percent of the constraints imposed by the rules-of-thumb interpretation procedures.

Keywords: *Geoeolectric; VES; traverse; groundwater; aquiferous*

RESUMO

Introdução: Um cliente solicitou que o grupo de estudos ajudasse a determinar locais adequados para um regime de perfuração em seu lote, localizado em uma elevação rochosa na encosta superior da Propriedade Lawu, Minna, Nigéria. **Objetivos**: O objetivo deste estudo é realizar um levantamento com propósito específico para identificar os melhores locais em uma propriedade edificada na sofisticada Propriedade Lawu que sejam adequados para um regime de perfuração voltado ao consumo doméstico. **Métodos**: A área de estudo foi reconhecida pela equipe de levantamento a fim de georreferenciar os locais que seriam ocupados para o levantamento de sondagem elétrica vertical no lote de 30 m x 20 m. Devido à extensa edificação neste lote,

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apenas uma travessia de quatro pontos ao longo da travessia de 30 metros da fachada do edifício foi demarcada na direção nordeste, limitando assim o desejo da equipe de levantamento de definir uma grade de levantamento apropriada. O padrão de aquisição de dados nas 4 x 1 estações de levantamento da travessia-fachada do lote seguiu a sequência "tradicional" de medições de arranjo Schlumberger, a partir da qual a equipe de levantamento progrediu com espaçamento de eletrodos de corrente em cada extremidade de um ponto de levantamento localizado nesta travessia-fachada visando uma profundidade máxima de levantamento de 100 m. Resultado: O conjunto de dados de sondagem elétrica vertical adquirido para este estudo foi registrado em planilhas de dados com propósito específico. **Discussão**: Baseando-se em procedimentos empíricos de regras práticas para interpretação de dados de sondagem elétrica vertical na província geológica do Complexo Cristalino Nigeriano, inferências dedutivas foram extraídas em relação apenas à Estação 4 de sondagem elétrica vertical sobre localização "assegurada" de água subterrânea e localização "fortemente aquífera". **Conclusão**: Assim, recomenda-se que a Estação VES 4 seja explorada no programa de perfuração planejado do cliente, especialmente porque este ponto de levantamento atende 100% das restrições impostas pelos procedimentos de interpretação por regras práticas.

Palavras-chave: *Geoelétrico, SEV, travessia, água subterrânea, aquífero.*

1. INTRODUCTION

A client who owns a limited-extent real estate property at the upmarket Lawu Estate requires information of pinpoint accuracy to enable him to determine the best location to invest in drilling a potable-water borehole, albeit after his lot has undergone extensive built-up development. The challenge offered by the client becomes the heart of the problem that the survey crew of this project must solve in light of the fact that, apart from the reality that the lot is already built up, the property is located at an upslope rocky knoll of this estate. This study aims to carry out a purpose-specific survey to pinpoint the best location in a built-up property at the upmarket Lawu Estate that would be suitable for a drilling regime targeted for household consumption. The specific objectives herein are employing the technique of the vertical electrical sounding (VES) mode of the geoelectrical method to achieve this aim in the dual formats of generation of log-log plots and their analyses thereof and generation of an accompanying pseudosection plot and its analysis thereof. The designated area of interest here, a 30 m x 20 m lot located at an upslope rocky knoll, was heavily built-up; thus, the survey crew designated a four-station traverse segmented into 1 x 4 = 4 VES survey stations at the frontage of the built-up compound. The VES survey regime was planned for a total depth of 100 m at each survey location. The wider study area of Lawu Estate is shown in the satellite imagery map of Figure 1. On this map, the built-up area forming the backdrop of the study area has been appropriated demarcated based on the georeferenced information of its edges: Point A at 09°39′50.6″;006°30′50.3″, Point B at 09°39′49.7″;006°30′49.0″, Point C 09°39′49.9″;006°30′49.8″, Point D at 09°39′50.7″;006°30′49.8″. Also indicated in Figure 1 are the four individual points along a linear stretch of the frontage earmarked for the survey (alas, the plotted solid red red triangles for the frontage-traverse are slightly skewed laterally in the northeasterly direction).

Figure 1*. The satellite imagery map shows the under-construction and partially built-up Lawu Estate, with the core study area appropriately demarcated by solid red triangles.*

According to Obaje (2009), Nigeria's geology is comprised of three major litho-petrological components: the basement complex, younger granites, and sedimentary b*asins*. The Basement Complex, which is Precambrian in age, is made up of the *Migmatite-Gneiss Complex*, the *Schist Belts,* and the *Older Granites.* The Younger Granites comprise several Jurassic magmatic ring complexes centered on Jos and other parts of northcentral Nigeria. They are structurally and petrologically distinct from the Older Granites. The Sedimentary Basins, containing sediment fill of Cretaceous to Tertiary ages, comprise the Niger

Delta, the Anambra Basin, the Lower, Middle, and Upper Benue Trough, the Chad Basin, the Sokoto Basin, the Mid-Niger (Bida-Nupe) Basin and the Dahomey Basin.

The Basement Complex forms a part of the Pan-African mobile belt and lies between the West African and Congo Cratons and south of the Tuareg Shield (Black, 1980). It is intruded by the Mesozoic calc-alkaline ring complexes (Younger Granites) of the Jos Plateau and is unconformably overlain by Cretaceous and younger sediments. The Nigerian basement was affected by the 600 Ma Pan-African orogeny, and it occupies the reactivated region which resulted from plate collision between the passive continental margin of the West African Craton and the active Pharusian continental margin (Burke and Dewey, 1972; Dada, 2006). The basement rocks are believed to be the results of at least four major orogenic cycles of deformation, metamorphism, and remobilization corresponding to the Liberian (2,700 Ma), the Eburnean (2,000 Ma), the Kibaran (1,100 Ma), and the Pan-African cycles (600 Ma). The first three cycles were characterized by intense deformation and isoclinal folding accompanied by regional metamorphism, which was further followed by extensive migmatization. The Pan-African deformation was accompanied by a regional metamorphism, migmatization, and extensive granitization and gneissification, which produced syntectonic granites and homogeneous gneisses (Abaa, 1983). Late tectonic emplacement of granites and granodiorites and associated contact metamorphism accompanied the end stages of this last deformation. The end of the orogeny was marked by faulting and fracturing (Gandu *et al*., 1986; Olayinka, 1992).

According to Yaman *et al.* (2020), the Minna Area is mainly underlain by rocks belonging to the Basement Complex. The authors observed that the main lithological unit that underlies the area is the granites (over 90%), virtually covering the entire map, whilst the other lithological unit is the schist, which occurs in the southeastern part of the area. The cross-section indicates a terrain that is not very rugged but gentle, with the schists forming at higher elevations. The elevation ranges from 240 m to 300 m, the highest point occurring around the Police Secondary School area. The authors remarked that the granites are the youngest of the two rock types.

Jonah *et al.* (2013) were tasked to locate an aquifer at a lot at the Dan Zaria Academic Estate, opposite the Gidan Kwano Campus, Federal University of Technology, Minna. The team members adopted a different approach from the conventional in order to do reconnaissance for the planned survey at this estate; the resistivity type of geoelectrical survey in the VES mode of the Schlumberger array was employed for the reconnaissance and final stages of this investigation. This “unconventional” approach was the acquisition of VES data at shallow depths (i.e., progressively down to 10m) over the area of study in order to determine the point of lowest resistivity instead of the approach to determine the lateral variation of resistivity at these shallow depths using the constant separation traversing (CST) method. The point of lowest resistivity thus identified was surveyed to a final depth of 100m. The authors observed that the 30-40m depth interval at this point was the possible groundwater yield zone.

In fidelity to the “conventional” approach, Jonah *et al.* (2014[a]) prospected for an aquifer at a lot located on a granitic knoll in Minna. At the outset, the client’s property was visually reconnoitered; the extent and preferred traverse directions were noted. The survey crew proposed a north-south (i.e., longitudinal traverse, LT) profiling scheme at a 10 m separation between survey stations and a 10 m separation between profile lines for the constant separation traversing reconnaissance phase to a depth of 15 m. Then, detailed vertical electrical sounding surveys were conducted for locations of “low-ohmic interest” to a depth of 100 m. The result of the reconnaissance phase indicated the lowest resistance value of 1.6348 Ω at “LT4-1.” Upon final VES surveys, it was concluded that the prospect for aquifers of good yield at the area of study was very poor indeed: this conclusion actually corroborated the one drawn from the initial survey that the crew was unaware of.

A third approach in the series of surveys undertaken by Jonah *et al.* (2014[b]) was the “No CST” format, informally described as “not carrying out any reconnaissance survey in order to determine the lateral variation of resistivity.” For this field technique, the VES survey to the 100 m-depth is carried out for each of the selected locations. The survey crew adopted this method to prospect for the suitable location for a desired borehole at a built-up compound of an enclosed bungalow with a self-contained sewage system; only the three corners of the brick fence away from the corner where the cesspool was situated defined in one north-south (longitudinal traverse, LT) and two east-west (transverse traverse, TT) modes were suitable for this survey. Since the client desired a borehole to be drilled at her property, the survey crew considered it

inexpedient to do a CST survey, hence the “No CST” format. The derived continuous variation of resistivity with depth model indicated that a four-layer sequence was identified for VES TT1, a four-layer sequence for VES LT1, and a three-layer sequence for VES TT2. The authors based their interpretation of aquifer prospects at the three VES locations on a combination of informal and fairly successful dual empirical rules to determine the likely presence of groundwater in the basement complex geological province. Based on these rules, TT1 indicates the best prospect for groundwater yield in the area of the survey with good showings from the 30m-depth mark down to the 50 m-depth; LT1 could be discounted in water yield terms with respect to TT1, and TT2 satisfies the criteria at the 20m-depth mark and discontinuously still, at the 50 m-depth mark before a spike in “ohmic” values. Also, it was observed that the 50 m-depth mark for TT1 and TT2 correlate very well as a prospective aquifer zone. If drilling must be done at all, then it was recommended that point TT1 be considered a good prospect for groundwater yield over the 20-m “yield window.” Because of the smoothly changing continuum of resistivity values down to the 100 m-depth mark, it was recommended that drilling should be terminated at this maximum or total depth (TD) of the survey in order to tap into the fractured basement at this TD. Incidentally, TT1 was upslope of the sewer pit, which was a plus for this VES station over the possible prospect of TT2.

Ibeneme *et al.* (2014) remark that the different aquifer units within the Lower Orashi River Sub-Basin, Southeastern Nigeria, were delineated using the Vertical Electrical Sounding (VES) technique. The authors observed that twenty-two (22) VES soundings were carried out using the ABEM SAS 4000 Terrameter. The data generated were analyzed using Zohdy software, which outputs modeled curves in terms of depth and resistivity. According to the authors, six profiles were taken in the northeast-southwest and northwest-southeast directions to cover the entire area of study. Four to six geoelectric layers comprising the topsoil, clayey sand, dry sandstone, saturated sandstone, shaley sand, and sandy shale were delineated, with the latter usually occurring as the last layer. The third and fourth layers underlying dry sandstone form the aquiferous unit. This unit was found to have an average resistivity value range of 10.7 – 6060 Ωm and an average thickness of 32 m. It was observed that most of the aquifer units within the area are unconfined, with static water levels varying between 10.6 to 62.8 m. Some of the aquifer units are shallow, with a static water level of less than 40 m, while others are deeper, with a static water level occurring over 60 m below the surface. It was advised that care ought to be taken in drilling and casing at shallow aquiferous areas to maintain proper sanitary conditions so as to reduce the risk of groundwater contamination.

Bahri *et al.* (2016) observe that their endeavor was dedicated to evaluating the quality of groundwater and associated pollution of aquifers at Sukolilo, Surabaya, East Java, Indonesia. They pointed out that the vertical electrical sounding procedure is a geoelectric method used to measure the resistivity of the rocks, and the associated instrumentation of this procedure is used to obtain subsurface information about aquifer depth. The authors employed a water quality tester to determine acidity, conductivity, salinity, oxidation-reduction potential, and total dissolved solid parameters. The authors reported that the prevailing aquifer thickness in the study area is in the region of 3.17 m, and the depth range is between 0.45m and 3.62 m. They also pointed out that local lithology is of an alluvium nature, and this changes toward the north, as indicated by the different depths of the observed rock layers. The authors mention that seawater has intruded into the groundwater at the Sukolilo area, the fact corroborated by high salinity and high total dissolved solid showings. Thus, the authors concluded that water from the unconfined aquifer in Sukolilo was polluted and not suitable for consumption.

Asta and Prasetia (2020), at the MATEC Web of Conferences 331, discussed the application of vertical electrical sounding with a resistivity meter based on a boost converter to estimate the potential of groundwater aquifers in Karang Anyar of Tarakan City, Indonesia. The authors noted that the vertical electrical sounding method is a route that can be used to predict geological and hydrogeological conditions. The authors observed that as a result of the investigation using a resistivity meter based on a boost converter, their result indicated the presence of groundwater at a depth of 7.91m to 44.33 m with a characteristic resistance value of 27.22 Ωm for the estimated lithology of sand.

Pacheco et al. (2023) explained that, in recent years, the occurrence of unexpected meteorological events during the dry season and population growth generated shortages in the supply of drinking water in the city of Pampas, Peru. This situation prompted the authors to look

for new search strategies for natural water sources, including underground sources.

The authors observed that faced with this problem, the possibility of detecting and parameterizing these sources was raised, while the design of a tubular well that allows the economic extraction of water from the aquifer was also studied; both of these objectives were achieved through the use of geophysical techniques, generating profile images of geological maps of the strata and the location of the possible water table of the study area.

The authors pointed out that the preferred locations for locating groundwater collections are alluvial fans and fractured valley bottoms. Using the Schlumberger array, eleven (11) vertical electrical soundings were completed up to a depth of 150 m. The acquired resistivity values vary between 6.32 Ωm and 125.23 Ωm. The PQWTS-150-Water Detector equipment was also used to measure the depth of the semi-confined aquifer and to know its groundwater flow.

The authors further noted that the profile of the geological map was described, and in this profile, clayey, silty, sandy, gravelly soils and a combination of them were found. Of particular interest, according to the authors, was VES Point 11, which was surveyed at the location of the nursery in the Daniel Hernández district, the area being flat and humid.

The authors noted that the aquifer at VES Point 11 has good hydrogeological possibilities that made surface recharge possible. The water table was also determined to be between 4 m and 8 m. Subsequently, the tubular well was designed. The authors concluded that the well was designed for a total depth of 115 m.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Hand-Held Global Positioning System (GPS) Unit

The hand-held Garmin GPSmap78® global positioning system unit shown in Figure 2 was employed to georeference the four corners of the lot to be surveyed as well as the individual locations selected for occupation for the VES data acquisition scheme.

***Figure 2**. Hand-held Garmin GPSmap78® global positioning system unit*

2.1.2 Resistivity Meter

The resistivity meter employed for the survey herein, the locally-built Vineyard Geological Survey brand, is shown in Figure 3.

***Figure 3**. A resistivity meter was employed for the survey*

2.2 Methods

2.2.1 Study Area Segmentation

At the outset, the study area was reconnoitered by the survey crew in order to georeference, in a preferred grid format, the locations that would be occupied for the VES survey in the 30 m x 20 m lot. Owing to the extensive build-up at this lot, only a four-point traverse along the 30-metric dimension traverse of the frontage of the building was demarcated in the north-westerly direction, thereby limiting the desire of the survey crew to define an appropriate survey grid. The defined four-point traverse along the frontage of the property becomes desirable in view of the fact that the resulting survey locations will

be as far separated from the household below-ground walled septic tank installation as possible. The four-point traverse is indicated in Figure 1.

2.2.2 The VES Survey

The VES data-acquisition pattern at the 4 x 1 (4) survey stations of the frontage traverse of the lot followed the "traditional" sequence of Schlumberger array layout measurements, whence the survey crew progressed with current-electrode spacing on either end of a survey point located at this frontage traverse targeting a maximum survey depth of 100 m. The frontage traverse on the shoulder of an inter-estate roadway means that there was no barrier to the desired progression of the field crew's measurement routine.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Generation of Log-Log Plots

Usually, after determining the resistivity values from the field resistance values, it is desirable to generate curves, commonly log-log plots, showing the variation of resistivity values with the total depth surveyed at that particular sequence for each VES station. It is recognized that the effective depth of penetration is equal to half the current electrode spacing (if distance AB separates the current electrodes, then this AB/2). According to Zohdy (1989), a continuous variation of resistivity with a depth curve is easily derived from a multilayer step-function curve by drawing a curve that passes through the logarithmic midpoint of each vertical and horizontal line on the multilayer step-function model. In view of the fact that the layer depths are logarithmically closely shaped, the derived continuous variation of resistivity with the depth model is equivalent to the original model. This approach makes it easy to construct maps of contoured resistivity values at different depths and contoured geoelectric sections. The field resistivity values were initially subjected to the log-log plot routine of the Windows-compatible WinResist® software, whence corresponding field curves for all the stations occupied were produced. The initial outputs were the "default" graphs. These were further smoothed by iterations, which were done in layers, thus resulting in final "modeled" outputs. The smoothed graphs are those that have connections to all the plotted points on the graph, and these are presented in Figures 4 to Figure 7. The log-log plots of VES Stations 1, 2, 3, and 4, presented as Figure 4, Figure 5, Figure 6, and Figure 7, have been conveniently (though erroneously labeled as those of VES Stations 13, 14, 15, and 16 which do not exist in the archive). Each of the WinResist® log-log plots provides information on the number of layers, the average resistivity values of these layers, their depths of occurrence, and their approximate thicknesses.

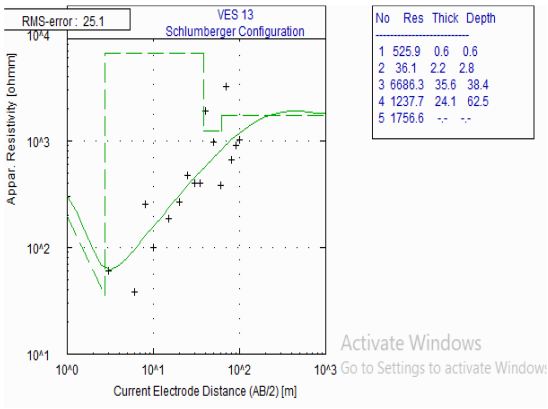

***Figure 4**. Log-log plot of VES Station 1 (erroneously labeled as "VES 13")*

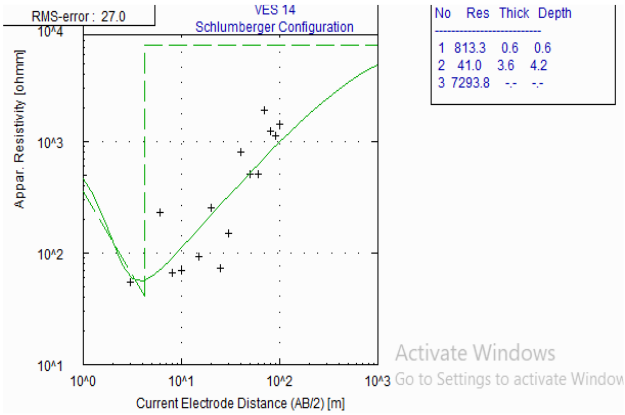

***Figure 5**. Log-log plot of VES Station 2 (erroneously labeled as "VES 14")*

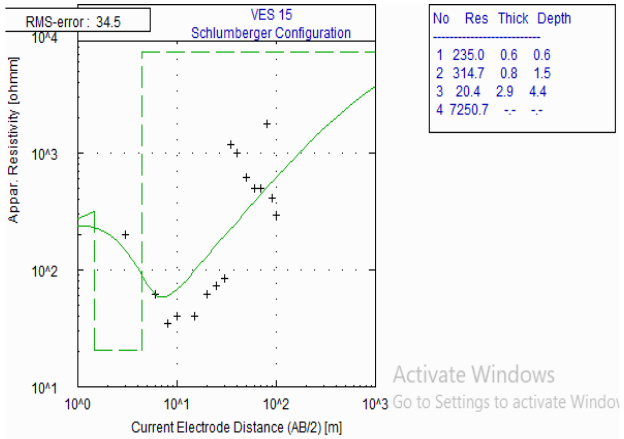

***Figure 6**. Log-log plot of VES Station 3 (erroneously labeled as "VES 15")*

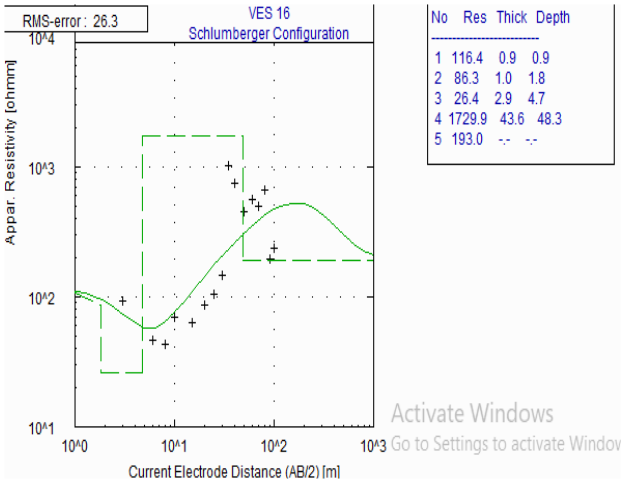

***Figure 7**. Log-log plot of VES Station 4 (erroneously labeled as "VES 16")*

3.1.2 Production of Pseudosection Plot

In order to show the resistivity cross-section along the traverse line of a survey at the study area, a pseudosection plot was generated, and this is shown in Figure 8.

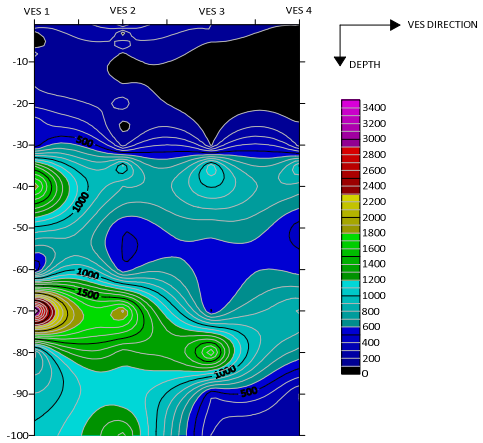

***Figure 8**. Pseudosection plot of study area*

3.2 Discussions

3.2.1 "Tricks" for Groundwater Search at the Nigerian Basement Complex

There exists, for the Nigerian Basement Complex, empirical rules (as complements to the "traditional" interpretation sequences that are taught in schools) by which workers can reliably make deductions with respect to the presence of sustainable groundwater at VES survey points along a line or across an area of study. Loke (2001) quoted Acworth (1981) as stating that "the weathered layer is thicker in areas with fractures in the bedrock." Jonah and Jimoh (2016) examined the validity of an empirical rule for delineating aquifer prospects at the Gidan Kwano Campus Development Phase II, Federal University of Technology, Minna, and this reliable route has been christened "Geoexplore Empirical Standardisation for Minna Area;" the Geoexplore Empirical Standardisation for Minna Area states that resistivity values between 200 Ωm and 300 Ωm at the 20 m depth and less than 200 Ωm at depths greater than 20 m are indicative of possible groundwater prospects. These recognized empirical rules, the veritable "tricks" for groundwater search at the Nigerian Basement Complex, are at the complementary core of the rules-of-thumb employed to determine the location of groundwater in the first and the fourth stage interpretation instances schedule, according to Jonah (2024).

3.2.2 The Log-Log Plots

The log-log plots indicate that, along the four stations of the traverse line of the survey, there is only one station designated as a three-layer location, only one designated as a four-layer location, whilst two stations are designated as five-layer locations. According to Olasehinde (personal communication), the three-layer structure is the expected norm in the local basement geological province. Whereas, too, the norm is to have a comparatively high resistivity value for the third layer of a discerned three-layer geological structure at a general area of survey at the local basement complex geological province in ordinal format, this condition is satisfied for VES Station 2 albeit not in ordinal format. The mid-section layers of the inferred four-layer locations of Figure 6 corresponding to VES Station 3 could be "compressed" into a single layer in order to achieve fidelity to the three-layer specification at the local geological province. In a similar vein, the mid-section layers of the inferred five-layer locations of Figure 4 and Figure 7 corresponding to VES Station 1 and VES Station 4 could be "compressed" into a single layer.

3.2.3 Fidelity to the Log-Log Plots of the First Rule-of-Thumb Schedule to Determine a Groundwater Location

The first rule-of-thumb is recognized herein to be the "traditional" or the "desired outcome" interpretation schedule whence the resistivity of the third layer in a three-layer geological sequence suddenly drops to the below-1000 Ωm values that indicate the presence of fracture for where the prevailing resistivity values of the second layer in this sequence are greater than the 1000 Ωm values that correspond to those for fresh

basement.

3.2.3.1 VES Station 1

VES Station 1 is not in fidelity to the first rule-of-thumb schedule.

3.2.3.2 VES Station 2

VES Station 2 is not in fidelity to the first rule-of-thumb schedule.

3.2.3.3 VES Station 3

VES Station 3 is not in fidelity to the first rule-of-thumb schedule.

3.2.3.4 VES Station 4

VES Station 4 is in fidelity to the first rule-of-thumb schedule.

3.2.4 Fidelity to the Log-Log Plots of the Second Rule-of-Thumb Schedule to Determine a Groundwater Location

The second rule-of-thumb is recognized herein to be the protocol of Acworth (1981), henceforth called the Acworth Protocol.

3.2.4.1 VES Station 1

VES Station 1 is not in fidelity to the second rule-of-thumb schedule.

3.2.4.2 VES Station 2

VES Station 2 is not in fidelity to the second rule-of-thumb schedule.

3.2.4.3 VES Station 3

VES Station 3 is not in fidelity to the second rule-of-thumb schedule.

3.2.4.4 VES Station 4

VES Station 4 is not in fidelity to the second rule-of-thumb schedule in a strict sense unless the *circa* 4.7 m thickness of the assumed second layer is considered relatively "thick."

3.2.5 Fidelity to the Log-Log Plots of the Third Rule-of-Thumb Schedule to Determine a Groundwater Location

The third rule-of-thumb is recognized herein to be the Geoexplore Empirical Standardisation for Minna Area.

3.2.5.1 VES Station 1

From examination of the corpus of data collected for this study, VES Station 1 is not in fidelity to the third rule-of-thumb schedule.

3.2.5.2 VES Station 2

From examination of the corpus of data collected for this study, VES Station 2 is not in fidelity to the third rule-of-thumb schedule by and large.

3.2.5.3 VES Station 3

From examination of the corpus of data collected for this study, VES Station 3 is not in fidelity to the third rule-of-thumb schedule by and large.

3.2.5.4 VES Station 4

From examination of the corpus of data collected for this study, VES Station 4 is in fidelity to the third rule-of-thumb schedule by and large, especially at the depth regimes of 20–30 m mark and at the greater depth of 90 m and beyond.

3.2.6 The Pseudosection Plot

The pseudosection plot of the four-station traverse-line study area shows discernible three-resistivity layers, the predominantly low-resistivity trend generally occurring at shallower than the 30 m depth but discernible at greater depth regime at especially the fourth VES station, although there exists a low-resistivity zone of interest at depth beneath the first VES station. The next high-resistivity trend predominates across the traverse line from the 30 m depth downwards, whilst the highest resistivity trend of unmistakable fresh basement character seems to "juts" from VES Station 1 at the 70 m to 80 m depth to seemingly terminate at VES Station 3.

4. CONCLUSIONS

4.1 The Log-Log Plots

4.1.1 Fidelity to the Log-Log Plots of the First Rule-of-Thumb Schedule to Determine a Groundwater Location

4.1.1.1 VES Station 1

As per the constraint of the first rule-of-thumb schedule to determine a groundwater location, VES Station 1 is not "hydro-centric." The

term “hydro-centric” is used herein to indicate “water-bearing.”

4.1.1.2 VES Station 2

As per the constraint of the first rule-of-thumb schedule to determine a groundwater location, VES Station 2 is not “hydro-centric.”

4.1.1.3 VES Station 3

As per the constraint of the first rule-of-thumb schedule to determine a groundwater location, VES Station 3 is not “hydro-centric.”

4.1.1.4 VES Station 4

VES Station 4 is tagged “hydro-centric” based on its conformity to the first rule-of-thumb schedule.

4.1.2 Fidelity to the Log-Log Plots of the Second Rule-of-Thumb Schedule to Determine a Groundwater Location

4.1.2.1 VES Station 1

As per the constraint of the second rule-of-thumb schedule to determine a groundwater location, VES Station 1 is not “hydro-centric.”

4.1.2.2 VES Station 2

As per the constraint of the second rule-of-thumb schedule to determine a groundwater location, VES Station 2 is not “hydro-centric.”

4.1.2.3 VES Station 3

As per the constraint of the second rule-of-thumb schedule to determine a groundwater location, VES Station 3 is not “hydro-centric.”

4.1.2.4 VES Station 4

As per the constraint of the second rule-of-thumb schedule to determine a groundwater location, VES Station 4 meets the “hydro-centric” classification under the “relaxed” condition of assuming that a 4.7 m thickness regime of the assumed second layer is relatively “thick.” Moreover, the “hydro-centric” nature and designation of VES Station 4 is assured from fidelity to the first rule-of-thumb schedule.

4.1.3 Fidelity to the Log-Log Plots of the Third Rule-of-Thumb Schedule to Determine a Groundwater Location

4.1.3.1 VES Station 1

As per the constraint of the third rule-of-thumb schedule to determine a groundwater location, VES Station 1 is not “hydro-centric.”

4.1.3.2 VES Station 2

As per the constraint of the third rule-of-thumb schedule to determine a groundwater location, VES Station 2 is not “hydro-centric.”

4.1.3.3 VES Station 3

As per the constraint of the third rule-of-thumb schedule to determine a groundwater location, VES Station 3 is not “hydro-centric.”

4.1.3.4 VES Station 4

VES Station 4 is tagged “hydro-centric” based on its conformity to the third rule-of-thumb schedule.

4.1.4 Percentage Fidelity of VES Station 1 to the Three Rules-of-Thumb: 0%.

4.1.5 Percentage Fidelity of VES Station 2 to the Three Rules-of-Thumb: 0%.

4.1.6 Percentage Fidelity of VES Station 3 to the Three Rules-of-Thumb: 0%.

4.1.7 Percentage Fidelity of VES Station 4 to the Three Rules-of-Thumb: 100%.

4.1.8 Deductive Inference Regarding VES Station 1

Based on percentage fidelity to the three rules-of-thumb, VES Station 1 is not a groundwater location.

4.1.9 Deductive Inference Regarding VES Station 2

Based on percentage fidelity to the three rules-of-thumb, VES Station 2 is not a groundwater location.

4.1.10 Deductive Inference Regarding VES Station 3

Based on percentage fidelity to the three rules-of-thumb, VES Station 3 is not a groundwater location.

4.1.11 Deductive Inference Regarding VES Station 4

Based on percentage fidelity to the three rules-of-thumb, VES Station 1 is an “assured” groundwater location or a “strongly aquiferous” location, “aquiferous” being a term coined by Jonah (personal communication) to indicate

association with the likelihood of aquifer in the subsurface.

4.2 The Pseudosection Plot

The pseudosection plot validates the “assured” groundwater location and “strongly aquiferous” location deductive inference drawn with regard to VES Station 4. The reason for not designating VES Station 1, VES Station 2, and VES Station 3 as “aquiferous” locations is obvious from the pseudosection plot.

A 75% “fail” margin for this survey does not come across as a surprise because of the location of the traverse line of the survey in an area of prominent and profuse granitic outcrop showings. In spite of the high 75% “fail” margin for this survey, VES Station 4 “checks off” all the constraints of the groundwater-determination rules-of-thumb for 100% assurance. Thus, it is strongly recommended that VES Station 4 be exploited in the planned drilling programme of the client.

5. DECLARATIONS

5.1 Study Limitations

No limitations were known at the time of the study.

5.2 Acknowledgements

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5.4 Competing Interests

The authors declare that there exists no conflict of interest whatsoever arising from the preparation of this manuscript for publication with any other competing interests, whether they be of the authors’ or of second parties and third parties thereof. The data employed in the enunciation of the textual material herein are original, having been duly acquired by the authors as part of the annual undergraduate schedule of project supervision here at the Federal University of Technology, Minna, Nigeria. This body of data field, duly archived for validation and reference purposes, is available for integrity checks anytime.

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Forum Article

THE REVOLUTION IN AMERICAN PUBLIC HEALTH POLICY: PETROLEUM-BASED DYES AND THE CHRONIC DISEASE EPIDEMIC

A REVOLUÇÃO NA POLÍTICA DE SAÚDE PÚBLICA AMERICANA: CORANTES DERIVADOS DE PETRÓLEO E A EPIDEMIA DE DOENÇAS CRÔNICAS

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ABSTRACT

Background: The American food regulatory landscape has historically been influenced by industry interests, resulting in the widespread use of petroleum-derived synthetic food dyes banned in European countries. Chronic disease rates in American children have increased from 3% in the 1960s to approximately 60% currently, with annual healthcare costs reaching $1 trillion. The appointment of Robert F. Kennedy Jr. as Secretary of Health and Human Services marks a paradigmatic shift toward transparency and industry accountability in food safety regulation. **Aim**: This forum analysis examines Kennedy Jr.'s revolutionary approach to food safety regulation, particularly his confrontational stance against petroleum-based food additives exemplified by his statement, "*if they want to eat petroleum, they should add it themselves at home*" and evaluates the broader implications for American public health policy and global regulatory standards. Methods: Critical analysis of Kennedy Jr.'s public policy statements, examination of epidemiological data trends, and evaluation of proposed regulatory frameworks through content analysis of official speeches and policy declarations from the Department of Health and Human Services. **Results**: Kennedy Jr.'s administration targets the systematic elimination of synthetic food dyes through industry partnerships, scientific transparency initiatives, and restoration of rigorous research standards. His confrontational rhetorical approach, compared to Mike Tyson's boxing style, has generated unprecedented industry cooperation with food companies "calling almost daily" seeking compliance guidance. The strategy combines voluntary industry agreements with open-source information databases and enhanced FOIA access. **Discussion**: This confrontational rhetoric represents unprecedented directness in health policy communication, challenging decades of established regulatory practices. The approach prioritizes scientific transparency over diplomatic language, generating both media attention and voluntary industry engagement that traditional regulatory pressure failed to achieve. **Conclusions**: Kennedy Jr.'s revolutionary stance may establish new global standards for food additive oversight, prioritizing public health over commercial interests through evidence-based policymaking and industry accountability measures. This paradigm shift from reactive to preventive regulatory models could influence international food safety governance and restore American leadership in global health policy.

Keywords: *food safety, synthetic dyes, public health policy, chronic disease, regulatory reform*

RESUMO

Introdução: O cenário regulatório alimentar americano tem sido historicamente influenciado pelos interesses da indústria, resultando no uso generalizado de corantes alimentares sintéticos derivados de petróleo que são proibidos em países europeus. As taxas de doenças crônicas em crianças americanas aumentaram de 3% na década de 1960 para aproximadamente 60% atualmente, com custos anuais de saúde chegando a US$ 1 trilhão. A nomeação de Robert F. Kennedy Jr. como Secretário de Saúde e Serviços Humanos marca uma mudança paradigmática em direção à transparência e responsabilização da indústria na regulamentação da segurança alimentar. **Objetivo**: Esta análise de fórum examina a abordagem revolucionária de Kennedy Jr. para a

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regulamentação da segurança alimentar, particularmente sua postura confrontativa contra aditivos alimentares à base de petróleo exemplificada por sua declaração "*se eles querem comer petróleo, devem adicioná-lo em casa*", e avalia as implicações mais amplas para a política de saúde pública americana e padrões regulatórios globais. **Métodos**: Análise crítica das declarações de política pública de Kennedy Jr., exame de tendências de dados epidemiológicos, e avaliação de estruturas regulatórias propostas através de análise de conteúdo de discursos oficiais e declarações políticas do Departamento de Saúde e Serviços Humanos. **Resultados**: A administração de Kennedy Jr. visa a eliminação sistemática de corantes alimentares sintéticos através de parcerias com a indústria, iniciativas de transparência científica e restauração de padrões rigorosos de pesquisa. Sua abordagem retórica confrontativa, comparada ao estilo de boxe de Mike Tyson, gerou cooperação industrial sem precedentes com empresas alimentícias "*ligando quase diariamente*" buscando orientação para conformidade. A estratégia combina acordos voluntários da indústria com bancos de dados de informações de código aberto e acesso aprimorado via FOIA. **Discussão**: Esta retórica confrontativa representa uma franqueza sem precedentes na comunicação de políticas de saúde, desafiando décadas de práticas regulatórias estabelecidas. A abordagem prioriza transparência científica sobre linguagem diplomática, gerando tanto atenção da mídia quanto engajamento voluntário da indústria que a pressão regulatória tradicional falhou em alcançar. **Conclusões**: A postura revolucionária de Kennedy Jr. pode estabelecer novos padrões globais para supervisão de aditivos alimentares, priorizando a saúde pública sobre interesses comerciais através da formulação de políticas baseadas em evidências e medidas de responsabilização da indústria. Esta mudança paradigmática de modelos regulatórios reativos para preventivos poderia influenciar a governança internacional de segurança alimentar e restaurar a liderança americana na política global de saúde.

Palavras-chave: *segurança alimentar, corantes sintéticos, política de saúde pública, doença crônica, reforma regulatória.*

1. INTRODUCTION: A NEW KENNEDY EMERGES

As a non-American observer of United States politics, I typically follow only fragments of news through digital platforms, without pretensions of expertise in that country's complex political landscape. For years, the interventions of Senator John Kennedy caught my attention, known for his ironic and provocative comments. Paraphrasing imperfectly one of his remarks about the search for extraterrestrial intelligence - something like "…*they should stop looking for intelligent life on other planets and look right here.*." - one perceives a politician who uses sarcasm as a rhetorical tool to highlight systemic incongruencies.

However, a new protagonist from the Kennedy family emerges in the American public health scenario: Robert F. Kennedy Jr., recently appointed Secretary of Health and Human Services. In an unprecedented declaration, Kennedy Jr. directly confronted the food industry with an equally provocative statement: "…*if they want to add petroleum, if they want to eat petroleum, they should add it themselves at home. But they shouldn't be feeding it to the rest of us without our knowledge or consent*" (Kennedy Jr., 2025).

Watching this man speak reminds one of the good times of boxing matches. RFK Jr. resembles a fighter in the style of the old Mike Tyson, where fights were overwhelming and decided in the first rounds. Equivalently, his speeches are overwhelming and bombastic, with statements that hit the target directly without diplomatic detours. This position represents a paradigmatic change in the American regulatory approach to food additives and marks the beginning of an era of unprecedented transparency in food safety.

2. THE EPIDEMIOLOGICAL CATASTROPHE: NUMBERS THAT DEMAND ACTION

The statistics presented by Kennedy Jr. paint a disturbing portrait of American health that transcends political discourse and enters the realm of national emergency. The transformation from a society where only 3% of children had chronic diseases in the 1960s to one where 60% currently suffer from such conditions represents more than an epidemiological shift—it constitutes a civilizational crisis.

Consider the scope of this transformation: neurological disorders including ADHD, speech and language delays, tics, Tourette syndrome, narcolepsy, and autism spectrum disorder have exploded in prevalence. Autoimmune diseases once considered rare in children—juvenile diabetes, rheumatoid arthritis, lupus, Crohn's disease—now constitute a significant portion of pediatric practice. The metabolic dysfunction is equally alarming, with 38% of American teenagers presenting pre-diabetes, a condition that sets the stage for a lifetime of medical intervention.

The economic implications are staggering. Approximately $1 trillion annually—equivalent to the American military budget—is spent treating chronic diseases. This represents not merely a healthcare expenditure but a fundamental reallocation of national resources from productive capacity to disease management. Kennedy Jr.'s observation that "74% of American kids cannot qualify for military service" transforms this from a health issue into a matter of national security.

Perhaps most striking is the demographic specificity of these changes. American teenagers now exhibit testosterone levels equivalent to 68-year-old men, while girls reach puberty six years earlier than previous generations. These are not gradual evolutionary changes but rapid systemic disruptions that demand immediate investigation and intervention.

3. PETROLEUM IN OUR FOOD: THE CHEMICAL REALITY

Kennedy Jr.'s focus on petroleum-derived food dyes is neither hyperbolic nor politically motivated—it reflects a chemical reality that most consumers remain unaware of. The synthetic food dyes commonly consumed by Americans are indeed derived from petroleum and coal tar, representing a fundamental category error in food production: the incorporation of industrial chemicals into consumable products.

The major offenders include Red Dye 40 (Allura Red AC), the most widely used synthetic dye in American food products, found in everything from candies to breakfast cereals. Red Dye 3 (Erythrosine), already banned in cosmetics by the FDA, continues to color food products consumed by children. Yellow 5 (Tartrazine) and Yellow 6 (Sunset Yellow) permeate the American food supply in soft drinks, cereals, and condiments. Blue 1 (Brilliant Blue FCF) and Blue 2 (Indigo Carmine) create the artificial colors in sports drinks and candies that have become synonymous with childhood nutrition.

The European response to these substances provides a telling counterpoint to American regulatory practice. These dyes are either banned outright or require warning labels in European Union countries based on scientific evidence linking them to hyperactivity and behavioral problems in children. The regulatory divergence is not merely bureaucratic—it reflects fundamentally different approaches to the precautionary principle in public health.

Kennedy Jr.'s strategic approach recognizes both the scientific inadequacy of current safety assessments and the practical challenges of regulatory change. His acknowledgment that "there are shockingly few studies on food dyes" exposes a critical gap: these substances have been grandfathered into the food supply without the rigorous safety testing now required for new additives.

4. SUGAR AS POISON: CONFRONTING ADDICTION IN FOOD POLICY

Kennedy Jr.'s characterization of sugar as "poison" and his comparison to crack cocaine represents perhaps the most controversial aspect of his policy platform. Yet this rhetoric, however provocative, reflects an emerging scientific understanding of sugar's neurochemical effects and metabolic consequences.

The epidemiological evidence supporting this position is compelling. Pediatricians who historically encountered diabetes perhaps once in their entire careers now see the condition in one-third of their young patients. This represents not a gradual increase but an exponential explosion that correlates directly with changes in food processing and sugar consumption patterns.

The addiction comparison, while inflammatory, has scientific basis. Sugar consumption triggers dopamine release in brain reward centers through mechanisms similar to those activated by addictive substances. The food industry's awareness of these effects—and their strategic utilization in product development—raises ethical questions about informed consent in food consumption.

Kennedy Jr.'s policy approach acknowledges the practical impossibility of eliminating sugar while demanding transparency in its presentation to consumers. This represents a sophisticated understanding of the regulatory limitations inherent in democratic governance while maintaining pressure for industry accountability.

5. THE TYSON APPROACH: RHETORIC AS REGULATORY STRATEGY

The comparison between Kennedy Jr.'s communication style and Mike Tyson's boxing approach is more than metaphorical—it reflects a deliberate strategic choice in public health communication. Traditional health policy discourse, characterized by diplomatic language and incremental recommendations, has failed to

generate the urgency necessary for addressing the chronic disease epidemic.

Kennedy Jr.'s confrontational rhetoric serves multiple strategic purposes. First, it generates media attention and public engagement with issues that typically receive limited coverage. Second, it establishes negotiating positions that allow for meaningful compromise while maintaining substantive reform objectives. Third, it signals to industry stakeholders that the regulatory environment has fundamentally changed.

The effectiveness of this approach is already evident in industry response. Kennedy Jr. reports that food companies are "calling almost daily" seeking guidance on compliance, suggesting that his rhetoric has achieved what years of traditional regulatory pressure could not: voluntary industry engagement with substantive reform.

6. SCIENTIFIC TRANSPARENCY AS REVOLUTIONARY ACT

Perhaps the most significant aspect of Kennedy Jr.'s approach is his commitment to scientific transparency and the elimination of conflicts of interest that have historically compromised regulatory science. His promise to restore "gold standard science" and eliminate industry influence represents a fundamental challenge to established regulatory practices.

The systematic suppression of adverse research findings through financial conflicts of interest has been documented across multiple regulatory agencies. Kennedy Jr.'s plan to fund independent research through NIH initiatives, coupled with the complete restoration of Freedom of Information Act access, could fundamentally alter the scientific basis for regulatory decision-making.

This transparency initiative extends beyond academic research to practical consumer information. The development of open-source databases documenting food additive content, combined with support for scanning applications that provide real-time ingredient analysis, represents a technological solution to information asymmetries in food markets.

7. INDUSTRY RESPONSE: COOPERATION THROUGH CONFRONTATION

Kennedy Jr.'s confrontational approach has generated unexpected industry cooperation, as evidenced by his direct statements: "*We're getting food companies now...who are calling us almost every day, asking us, how do we do this? What do you want us to do?*" This factual observation of increased industry engagement contrasts sharply with traditional expectations of corporate resistance to regulatory reform.

The food industry's stated preference for regulatory consistency provides concrete evidence for their collaborative approach. Kennedy Jr. reported that companies explicitly told his administration: "*the worst thing for us, is, if we have a patchwork of legislation and all these different states...And they didn't want that. and that's one of the reasons they came to the table with us*." This direct testimony reveals industry motivations beyond simple compliance concerns.

An analytical interpretation suggests this cooperation reflects a sophisticated understanding of corporate decision-making processes, where uniform national standards become preferable to inconsistent state-by-state regulations. Companies may view federal regulatory requirements as potentially advantageous for market positioning, particularly given the increasing consumer pressure for cleaner ingredients.

Kennedy Jr. acknowledges some form of industry agreement, stating: "*I want to commend Food companies for working with us. To achieve this, this agreement or this settlement*." However, the specific details of these agreements remain unclear in his public statements.

His view of the industry's motivation comes across as genuinely hopeful: "*I think they're ready to change the industry. They have children too... and I believe most of them truly desire a healthier America*." Whether this perspective stems from direct interactions with industry insiders or will be validated by actual implementation outcomes is yet to be seen.

8. GLOBAL IMPLICATIONS: AMERICAN LEADERSHIP IN REVERSE

The international dimensions of Kennedy Jr.'s food safety revolution extend beyond domestic health outcomes to questions of American technological and regulatory leadership. For decades, American regulatory standards have influenced global practices through market size and technological innovation. The current situation reverses this dynamic, with European safety standards serving as the model for American reform.

This regulatory reversal reflects broader

questions about American institutional capacity and scientific leadership. Kennedy Jr.'s reforms, if successful, could restore American credibility in global health governance while providing a model for other nations grappling with similar chronic disease epidemics.

9. CONCLUSION: THE BATTLE FOR AMERICAN HEALTH

Kennedy Jr.'s declaration about petroleum-derived dyes symbolizes a revolution in American public health policy that extends far beyond individual additives to fundamental questions about the relationship between government, industry, and public welfare. His approach combines scientific urgency, regulatory transparency, and industry engagement in an unprecedented manner that could establish new global standards for food safety governance.

The success of this initiative depends not merely on scientific evidence or regulatory authority but on sustained public engagement and political will. Kennedy Jr.'s rhetorical strategy, however controversial, has succeeded in generating both. Whether this translates into lasting institutional change remains to be seen, but the paradigm shift is already evident.

This revolutionary approach represents more than policy reform—it constitutes a fundamental reassertion of public health priorities over commercial interests. In an era where chronic disease has become the dominant health challenge globally, Kennedy Jr.'s model may prove essential for other nations seeking to reclaim regulatory independence from industry influence.

The battle for American health has begun, and its outcome will resonate far beyond national borders.

10. DECLARATIONS

10.1. Study Limitations

This analysis is based on public statements and policy declarations, representing a contemporary assessment of ongoing regulatory changes rather than a retrospective evaluation of implemented policies.

10.2. Acknowledgements

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10.4. Competing Interests

The author considers RFK Jr.'s proposed actions in a positive light, which may introduce bias into this perspective.

10.6. AI Usage Declaration

AI tools, including Claude and Grammarly, were used to improve text quality, assist in text organization, and provide grammar reviews. All analytical content, interpretations, and conclusions remain the author's original work and responsibility.

11. HUMAN AND ANIMAL-RELATED STUDIES

This research utilized content analysis of publicly available material, specifically a public speech by Robert F. Kennedy Jr. regarding food safety policy. The work did not involve direct research with human or animal subjects, nor did it require primary data collection from individuals. The source material consists entirely of publicly accessible governmental communications and policy statements made by public officials in their official capacity. As such, no ethical approval was required for this analysis, and the standard human subjects research protocols do not apply to this manuscript. The research methodology involved only secondary analysis of publicly available information, similar to historical document analysis or media content analysis.

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Kennedy Jr., R.F. (2025). *FULL SPEECH: RFK Jr. on plans to discontinue petroleum-based synthetic food dyes*. Public speech. Available at: https://youtu.be/SWwclQ1dqnw?si=zLZ4Th0Y7wYGezsw

SOUTHERN JOURNAL OF SCIENCES

ESTABLISHED IN 1993

Interview

INTERVIEW WITH DEPUTY VICE CHANCELLOR DR. O. A. OMOTESHO, UNIVERSITY OF ILORIN, NIGERIA (ENGLISH VERSION)

ENTREVISTA COM O VICE-REITOR DR. O. A. OMOTESHO, UNIVERSIDADE DE ILORIN, NIGÉRIA (VERSÃO EM INGLÊS)

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The complete version of the interview is available at: https://youtu.be/yMm919grTvU?si=gPq-diNIBulEuKfC

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ABSTRACT

Background: The University of Ilorin, founded in 1975 in Nigeria, has evolved from 3 to 16 faculties, becoming the country's most sought-after institution for the past two decades. **Aims**: To document the institutional evolution, identify the most demanded programs, assess scientific output, examine internationalization strategies, and understand strategic development objectives. **Methods**: Structured interview with Vice-Chancellor Dr. Olubumi Abayomi Omotesho, following a standardized protocol covering historical, academic, scientific, and strategic aspects of the institution, under Creative Commons license format. **Results**: The university expanded to 16 faculties in 49 years. The most demanded programs are Medicine and Nursing, followed by Pharmacy, Law, Engineering, and Accounting. Areas with the highest scientific output: Medicine, Biological/Agricultural Sciences, and Engineering. It offers 340 postgraduate programs with approximately 7,523 students. There is a dedicated infrastructure for internationalization, with plans for international accommodations. Discussion: The predominance of healthcare courses reflects global employability trends. Research aligned with Sustainable Development Goals demonstrates a contemporary vision. The institutional goal (number one in Nigeria, top 10 in Africa, top 500 globally) shows a measurable strategic approach. Commitment to internationalization aligns with global education trends. **Conclusions**: The institution exemplifies an evolving African university focused on academic excellence, scientific relevance, and internationalization. The prioritization of student-centered development, clear positioning goals, and international collaboration initiatives establish solid foundations for its contribution to regional and global knowledge.

Keywords: *University of Ilorin; Nigerian Higher Education; Academic Internationalization; Institutional Development; African Scientific Production.*

RESUMO

Introdução: A Universidade de Ilorin, fundada em 1975 na Nigéria, evoluiu de 3 para 16 faculdades, tornando-se a instituição mais procurada do país nas últimas duas décadas. **Objetivos:** Documentar a evolução institucional, identificar programas mais procurados, avaliar produção científica, examinar estratégias de internacionalização e compreender objetivos estratégicos de desenvolvimento. **Métodos:** Entrevista estruturada com o Vice-Reitor Dr. Olubumi Abayomi Omotesho, seguindo protocolo padronizado abrangendo aspectos históricos, acadêmicos, científicos e estratégicos da instituição, sob formato de licença Creative Commons. **Resultados:** A universidade expandiu para 16 faculdades em 49 anos. Os programas mais demandados são Medicina e Enfermagem, seguidos por Farmácia, Direito, Engenharia e Contabilidade. Áreas com maior produção científica: Medicina, Ciências Biológicas/Agrícolas e Engenharia. São oferecidos 340 programas de pós-

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graduação com aproximadamente 7.523 estudantes. Há infraestrutura dedicada à internacionalização, com planos para alojamentos internacionais. **Discussão:** A predominância de cursos da área de saúde reflete tendências globais de empregabilidade. A pesquisa alinhada aos Objetivos de Desenvolvimento Sustentável demonstra visão contemporânea. A meta institucional (número um na Nigéria, top 10 na África, top 500 global) evidencia abordagem estratégica mensurável. O compromisso com internacionalização alinha-se às tendências de educação global. **Conclusões:** A instituição exemplifica uma universidade africana em evolução, com foco em excelência acadêmica, relevância científica e internacionalização. A priorização do desenvolvimento centrado no estudante, metas claras de posicionamento e iniciativas de colaboração internacional estabelecem bases sólidas para sua contribuição ao conhecimento regional e global.

Palavras-chave: *Universidade de Ilorin; Ensino Superior Nigeriano; Internacionalização Acadêmica; Desenvolvimento Institucional; Produção Científica Africana.*

1. INTRODUCTION

It is with great satisfaction that we present this interview conducted with Dr. Olubumi Abayomi Omotesho, Vice-Chancellor of the prestigious University of Ilorin, Nigeria. This unique opportunity allows us not only to better understand this important African academic institution, but also to celebrate its 50 years of existence and contribution to the educational development of Nigeria and West Africa. We sincerely thank the university administration, especially Dr. Olubumi Atolani, for his fundamental role in making this transcontinental dialogue possible, which strengthens international academic relations and promotes knowledge exchange.

Image: 50th anniversary celebration of the University of Ilorin – NG.

Interviewer: Today, we have the honor of receiving Dr. Olubumi Abayomi Omotesho, Vice-Chancellor of the University of Ilorin. I would also like to take this opportunity to thank Dr. Olubumi Atolani, who organized this meeting for us. Thank you.

Image: Dr. Olubumi Abayomi Omotesho

Now, I'm going to present some rules and disclosures that will allow all of our partners to have the same time to make a presentation. Rule number one: our interview will last about 30 minutes or less, correct?

Dr. Omotesho: Correct.

Interviewer: Rule number two: our interview will be distributed under a Creative Commons license so it can be shared across various platforms.

Dr. Omotesho: Correct.

Interviewer: Number three, all our questions are the same for all our partners. And number four, I am a professor, not a professional reporter, so this is a big limitation.

Dr. Omotesho: Correct.

Interviewer: So, Professor Omotesho, my first question is: what is the oldest course offered by the University of Ilorin, and when was it founded?

Dr. Omotesho: Thank you very much. The University of Ilorin was founded in August 1975, almost today, exactly 49 years ago. The university started with three faculties, so all the courses in those three faculties started more or less at the same time. We had the Faculty of Arts, Faculty of Sciences, and Faculty of Education. Those were the founding faculties. The university started 49 years ago.

Image: University of Ilorin Logo.

Interviewer: That's very good.

Dr. Omotesho: Let me add that we have grown from those three faculties to 16 faculties.

Interviewer: 16 faculties today?

Dr. Omotesho: 16 faculties today, yes.

Interviewer: That's impressive. Professor, which course is currently most sought after by students?

Dr. Omotesho: Interestingly, our admission process is happening now, and the most popular course that has the highest number of applicants is Medicine, the medical program, and also Nursing, which is also a medical program. I believe the attraction is the fact that it's a professional course and alumni from the faculty, in almost all continents, have high mobility, and their certificate is well accepted in other areas, making it very attractive for people who want to enter the University of Ilorin. I would also like to mention that consistently, over the past more than a decade, approaching two decades, Ilorin is the most preferred university in Nigeria in terms of number of applicants who want to enter the university. We consistently lead.

Besides medicine, we also have great demand for the MBBS program, which makes up the medical program, and nursing, which is related to medicine. We also have many applicants in Pharmacy, Law, Engineering, and Accounting. These are also highly sought-after programs.

Interviewer: But number one is Medicine, and number two is Nursing, correct?

Dr. Omotesho: Perfect.

Interviewer: So, third question. Which research areas have the highest number of scientific publications at the institution, and in which courses do they excel?

Dr. Omotesho: For this question, I consulted the Scopus database of publications, and the largest scientific publications related to the University of Ilorin are in Medicine, Biological and Agricultural Sciences, and Engineering. These have the highest number of scientific publications in the Scopus database.

Interviewer: That's very good. So, next question. Remember, I'm not a professional reporter. We have some limitations.

Dr. Omotesho: Yes. I need to add some information.

Interviewer: Yes, please.

Dr. Omotesho: Our Vice-Chancellor had a program when he took office almost two years ago with the goal of making the University of Ilorin number one in Nigeria.

Interviewer: Correct.

Dr. Omotesho: And within the top ten in Africa and top 500 in the world.

Interviewer: That's very impressive.

Dr. Omotesho: Many initiatives have been implemented to achieve this goal. We are encouraging clusters, research clusters aligned with the Sustainable Development Goals (SDGs), and we are encouraging researchers. There is a great effort in terms of fundraising from around the world to help fulfill this objective.

Interviewer: Perfect. Thank you. Now, question number four. How many graduate courses, such as master's and doctorate, does the University of Ilorin currently offer? And what are the areas of knowledge?

Dr. Omotesho: The University of Ilorin currently has 340 graduate programs distributed across the 16 faculties. This year, we normally recruit our doctoral students twice a year. We just finished one round of applications. We had a total of approximately 2,136 applicants for graduate programs, both master's and doctorate. The total number of graduate students we have on campus currently is approximately 7,523 in various programs.

The most popular is the MPH, the Master's in Public Health. The Public Health program is the most sought-after graduate program. We also have the Master's in Educational Management, which is highly requested. Next, we have the Master's in Computer Science and the Master's in Law. Perhaps because the Chancellor is a lawyer, this is also a very popular program (laughter).

Interviewer: (laughter) No preferences on this matter. (laughter)

Dr. Omotesho: Regarding the Doctorate, the most popular is the Educational Technology program. We also have a Doctorate in Development Studies, which is very popular. The third most sought after is educational management. In the medical area, professionals are more focused on their professional specialties, not so much on master's or doctorate. Advanced academic training is just beginning to be incorporated into medicine. They remain more focused on their professional career.

Interviewer: Yes, I understand. It makes sense. It's an excellent and demanding career. Now, professor, moving to my next question. When international students want to study at the University of Ilorin, what is the process from submission of students' applications to proposal until their arrival at the university? How does it work?

Dr. Omotesho: We have a Center for International Education that coordinates activities with the external environment. The center works very closely with the Graduate School. We have a Graduate School called the School of Postgraduate Studies. Interested students register on the university portal. These applications are processed by the Center for International Education. They normally interact with students and coordinate activities in terms of connection with the Graduate School and later with the departments.

Sometimes, some of the students may have identified potential supervisors they want to work with. Thus, a connection is established between the proposed supervisor and the prospective student. The Center for International Education, which we call CIE, plays a vital role in this connection. Most of our processes are automated. Actually, we are working now to improve our website and portal. I'm sure that before the end of this month, everything will be working properly. Practically all our activities are carried out digitally.

Interviewer: Perfect. So professor, my next question. How are the facilities and infrastructure designed to accommodate international students? Does the university offer housing or student residence for students? If so, what are these options like? How to apply?

Dr. Omotesho: As I mentioned, the University of Ilorin has consistently been Nigeria's most sought-after university. Last week, we received an award. We have an existing international accommodation. The Vice-Chancellor suggested, and the projects are already ready, that we start construction of what we call international student residences. Using the resources we received recently, the projects have been finalized, and construction will begin soon.

Our goal is for it to be an international standard of accommodation. And not exclusively for international students. We also want students to be able to interact with some of our local

students. We are planning a ratio of approximately 80 to 90 percent international students with about 10 percent local students, to avoid isolation. Implementation has already begun. Currently, we have a residence, but it doesn't offer enough space. This is one of the reasons for building the new accommodation.

The agency that organizes university admission for undergraduate programs in Nigeria is called the Joint Admissions and Matriculation Board. We decided, in recognition of the award received, to name this residence the Joint Admissions and Matriculation Board International Accommodation.

Interviewer: That's excellent. I would like to ask more questions, but I'll stick to the list to conduct a balanced interview, correct?

Dr. Omotesho: Correct.

Interviewer: If there's an opportunity, we'll conduct more interviews in the future.

Dr. Omotesho: Certainly.

Interviewer: Now, question number seven. The University of Ilorin is participating for the second time in the Southern Science Conference, which follows a unique organizational model involving multiple universities. What benefits does the university expect to provide to its students by engaging in this international event?

Dr. Omotesho: We are excited because when our students participate in this event, exposure opportunities expand considerably. Meeting people from diverse backgrounds represents a fundamental aspect of education. We hope they can keep up with the latest trends and connect with colleagues from around the world. The internet has transformed the world into a global community. However, going out and actually interacting with these people will certainly enhance their exposure and provide different perspectives from what they are accustomed to, particularly for those with limited international exposure previously.

We hope they can develop higher-quality research in terms of scope and discover opportunities they hadn't considered previously. We also believe they will be able to collaborate more effectively with people in areas where there are better infrastructures, contributing to their comprehensive training as students of excellence.

Interviewer: Perfect. Thank you very much.

Now, this is a very important question. Considering the challenges presented by our different time zones, what suggestions would you give to the event organizers to maximize participation from University of Ilorin students?

Dr. Omotesho: The time zone difference represents a significant challenge, but it's something for which there aren't many solutions. I believe interest is the determining factor. I've had occasions when I needed to wake up during the night to participate in virtual meetings. Interest is the fundamental element. One can always adjust schedules to check what would be the most suitable time for discussions, as we did when scheduling this conversation with Dr. Atolani. Considering my schedule, for example, I would have preferred a later time in Nigeria. Therefore, I don't consider time zones to be an insurmountable obstacle.

Interviewer: Very good.

Dr. Omotesho: Yes, because it's a circumstance over which we have no control.

Interviewer: Exactly, something we cannot change.

Dr. Omotesho: We can always adapt our schedules to enable productive interactions.

Interviewer: Thank you very much, Professor. And now we come to the last question. By participating in an international event at no cost to students, the university demonstrates its commitment to providing valuable opportunities. How do you evaluate the importance of student participation in international events for their academic and professional development?

Dr. Omotesho: Our students are extremely important to us. We always emphasize that without students, there would be no professors, vice-chancellors, deputy vice-chancellors, or any staff. All our activities are student-centered. We take immense pride in our students, a feeling they also share. Recently, I participated in the inauguration ceremony of the new student representatives, elected a few weeks ago.

One of the most important initiatives we aspire to, as I mentioned, is the Vice-Chancellor's goal, called "one 10 500" [reference to the goals of becoming number one in Nigeria, among the top ten in Africa, and among the top 500 in the world]. We want to provide our students with the greatest possible exposure to best practices in all aspects of their educational training. We recognize that this is crucial in today's globalized world. The greater the exposure provided to students, the greater their capacity for international mobility. Therefore, we seek to expand their horizons. The memoranda of understanding, interactions, and all our initiatives aim to enhance our students' training and the quality of our work.

Interviewer: You are constantly seeking the best for your students. We are looking forward to this exchange with diverse students from around the world. And we know that this will fundamentally contribute to the improvement of our students.

Dr. Omotesho: This is an excellent perspective.

Interviewer: Thank you very much, Professor. We conclude our interview. On behalf of my conference colleagues, I would like to express my sincere thanks for your time.

Dr. Omotesho: Thank you very much. It was a pleasure talking with you. I also thank our colleague, Professor Atolani.

Interviewer: Come greet him. He was just waiting to greet you.

Dr. Omotesho: He's here.

Interviewer: Hello, Professor. How are you?

Dr. Atolani: Thank you. Thank you very much.

Interviewer: Thank you for the opportunity to talk with all of you. I hope to see you again.

Dr. Omotesho: Certainly. In the next edition (2026), we hope to be the best presenters. In the last edition we came in second place.

Interviewer: You were already excellent in the previous edition. The best presentation is on the way.

Interviewer: I hope to see many students from Ilorin at the conference. We'll all meet in November.

Professor Atolani: Yes. Thank you. Thank you very much. Have an excellent day.

Interviewer: Likewise. Thank you.

This interview was part of the interinstitutional scientific dissemination partnership project of the SSCON – 2024 conference, continuing for the future 2026 edition in RJ.

Image: SSCON 2024 Logo.

DECLARATIONS

1. Limitations: The interview is limited to its content.

2. Funding source: The host funded this interview.

3. Conflicts of interest: The host has worked for the journal for many years and this may have influenced the interview.

SOUTHERN JOURNAL OF SCIENCES

ESTABLISHED IN 1993

Review paper

TREATMENTS FOR ACUTE LYMPHOBLASTIC LEUKEMIA: A COMPARISON BETWEEN TISAGENLECLEUCEL AND CLOFARABINE

TRATAMENTOS PARA LEUCEMIA LINFOBLÁSTICA AGUDA: UMA COMPARAÇÃO ENTRE TISAGENLECLEUCEL E CLOFARABINA

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ABSTRACT

Background: Acute lymphoblastic leukemia (ALL) is a heterogeneous hematological malignancy predominantly affecting individuals under 20 years of age. Traditional chemotherapy, such as clofarabine, has shown efficacy; however, novel immunotherapeutic strategies like tisagenlecleucel (Kymriah®) have significantly altered the treatment paradigm. **Aim:** This study aimed to perform a comparative analysis of tisagenlecleucel, a CAR-T cell therapy, and clofarabine, a second-generation purine nucleoside analog, evaluating their mechanisms of action, therapeutic benefits, limitations, and clinical applicability across diverse patient populations. **Methods:** A systematic comparative evaluation was conducted, encompassing pharmacological characteristics, mechanisms of action, treatment protocols, efficacy, safety profiles, and clinical indications of both agents. The analysis considered pharmacokinetic and pharmacodynamic data and included patient demographic variables. **Results:** Tisagenlecleucel demonstrated high efficacy in refractory B-cell ALL, with durable responses and a blood half-life of 128 days, but with notable immune-related adverse effects such as cytokine release syndrome. Clofarabine, effective across a broader patient population, acts via multiple antitumor mechanisms but carries significant toxicity risks, including infection and sepsis. **Discussion:** The therapies present distinct clinical profiles: tisagenlecleucel offers targeted immunotherapy with high specificity but requires specialized infrastructure and management of immune toxicities. Clofarabine is more widely accessible and applicable, but is associated with conventional chemotherapy-related side effects. Treatment accessibility and cost differ markedly between the two. **Conclusions:** Therapy selection should be personalized based on patient-specific factors and institutional resources. Tisagenlecleucel is ideal for pediatric and young adult patients with relapsed/refractory B-cell ALL in CAR-T-capable centers, while clofarabine remains a viable option for broader ALL populations, particularly when genetic therapies are not feasible. Further research is needed to optimize therapeutic strategies and improve access to advanced treatments.

Keywords: *tisagenlecleucel, clofarabine, Acute lymphoblastic leukemia.*

RESUMO

Introdução: A leucemia linfoblástica aguda (LLA) é uma neoplasia hematológica heterogênea que afeta predominantemente indivíduos com menos de 20 anos de idade. A quimioterapia tradicional, como a clofarabina, tem demonstrado eficácia; no entanto, estratégias imunoterapêuticas inovadoras como o tisagenlecleucel (Kymriah®) alteraram significativamente o paradigma de tratamento. **Objetivo:** Realizar uma análise comparativa

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do tisagenlecleucel, uma terapia com células CAR-T, e da clofarabina, um análogo de nucleosídeo de purina de segunda geração, avaliando seus mecanismos de ação, benefícios terapêuticos, limitações e aplicabilidade clínica em diversas populações de pacientes. **Métodos:** Foi conduzida uma avaliação comparativa sistemática, abrangendo características farmacológicas, mecanismos de ação, protocolos de tratamento, eficácia, perfis de segurança e indicações clínicas de ambos os agentes. A análise considerou dados farmacocinéticos e farmacodinâmicos e incluiu variáveis demográficas dos pacientes. **Resultados:** O tisagenlecleucel demonstrou alta eficácia na LLA de células B refratária, com respostas duradouras e meia-vida sanguínea de 128 dias, mas com efeitos adversos relacionados ao sistema imunológico notáveis, como a síndrome de liberação de citocinas. A clofarabina, eficaz em uma população de pacientes mais ampla, atua através de múltiplos mecanismos antitumorais, mas carrega riscos significativos de toxicidade, incluindo infecção e sepse. **Discussão:** As terapias apresentam perfis clínicos distintos: o tisagenlecleucel oferece imunoterapia direcionada com alta especificidade, mas requer infraestrutura especializada e manejo de toxicidades imunológicas. A clofarabina é mais amplamente acessível e aplicável, mas está associada a efeitos colaterais convencionais relacionados à quimioterapia. A acessibilidade ao tratamento e o custo diferem marcadamente entre os dois. **Conclusões:** A seleção da terapia deve ser personalizada com base em fatores específicos do paciente e recursos institucionais. O tisagenlecleucel é ideal para pacientes pediátricos e adultos jovens com LLA de células B recidivada/refratária em centros capazes de realizar terapia CAR-T, enquanto a clofarabina permanece uma opção viável para populações mais amplas de LLA, particularmente quando terapias genéticas não são viáveis. Mais pesquisas são necessárias para otimizar estratégias terapêuticas e melhorar o acesso a tratamentos avançados.

Palavras-chave: *tisagenlecleucel, clofarabina, leucemia linfoblástica aguda.*

1. INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a heterogeneous hematological malignancy characterized by the proliferation of immature lymphoid cells—specifically B-cell lymphoblasts in B-cell ALL. These malignant cells infiltrate the bone marrow, spread to the peripheral blood and other organs, disrupt normal hematopoiesis, and can lead to organ failure and death if left untreated (Agencia Española de Medicamentos y Productos Sanitarios, 2019).

Initially, clofarabine monotherapy was employed. This purine nucleoside analog interferes with DNA replication and RNA transcription in leukemic cells, thereby inhibiting their proliferation and inducing apoptosis. More recently, Kymriah® (tisagenlecleucel), a gene therapy involving genetically modified autologous T cells, has emerged as an alternative treatment.

This review compares the two therapeutic approaches in terms of their mechanisms of action, clinical advantages, and limitations, with the aim of evaluating their relative efficacy in the treatment of ALL.

2. METHODS

A systematic review of the literature in PubMed and Scielo was performed to search for publications describing the use tisagenlecleucel and clofarabine for the treatment of acute lymphoblastic leukemia, collecting and analyzing data. In order to do so, we used the following words/terms in combination: tisagenlecleucel AND treatments AND acute lymphoblastic leukemia. The exclusion criteria consisted of limiting papers on the use of any of those drugs from 2009 to 2023. The work was made as a task for the subject Biotecnología, belonging to the Pharmacy and Biochemistry career, and the extension and number of citations were restricted to the indication of the catherdra.

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1Tisagenlecleucel

3.1.1.1 Pharmacology

Tisagenlecleucel (Kymriah®) is a gene therapy involving T cells extracted from the patient. These cells are genetically modified ex vivo using a viral vector to express chimeric antigen receptors (CAR-T), enabling the T cells to recognize and destroy cells expressing the CD19 antigen. This includes both malignant and healthy B lymphocytes (1).

3.1.1.2 Treatment Process:

1. T-cell extraction: Blood draw followed by leukapheresis.
2. Genetic modification: Ex vivo enhancement of immune response.

3. Reinfusion: Modified T cells are reinfused to target cancer cells.

The therapy is indicated for pediatric and young adult patients up to 25 years old with refractory B-cell ALL (Agencia Española de Medicamentos y Productos Sanitarios, 2019).

3.1.1.3 Structure and Mechanism of Action

CAR-T receptors are produced by transfecting T cells with a lentiviral vector. The receptor's structure includes:

- **CD3-zeta signaling domain:** Essential for T-cell activation against tumor cells.
- **CD8-α transmembrane domain** (from human receptors) and **CD137 (4-1BB) costimulatory domain:** Enhance T-cell persistence and expansion in vivo.
- **Murine-derived single-chain variable fragment (scFv):** Binds to CD19, an antigen highly expressed on B lymphocytes in ALL patients (2,3).

CD19 regulates B lymphocyte proliferation and activation, making it a critical target in B-cell ALL.

3.1.1.4 Pharmacodynamics

After reinfusion, modified T cells interact with CD19, releasing antitumor cytokines and signaling the targeted cell. Cytokines also promote T-cell expansion and selectivity for CD19, increasing treatment efficacy and toxicity (1,3).

3.1.1.5 Pharmacokinetics and Metabolism

Tisagenlecleucel is administered intravenously, with immediate availability. Post-reinfusion, cellular expansion follows a biexponential decline due to tissue distribution. The average blood half-life is 128 days, depending on dose, proliferation, and cell viability. Dosage recommendations:

- Patients ≤50 kg: 0.2–2.5 × 10^6 or 0.1–2.5 × 10^8 transduced T cells.
- Patients >50 kg: 1.0–2.5 × 10^8 transduced T cells (1, 2).

3.1.1.6 Advantages

- Applicable to various cancers.
- Innovative therapy using modified T cells.
- High complete response rate (4, 5).

3.1.1.7 Disadvantages

- Adverse effects: cytokine release syndrome.
- Severe risks: exaggerated immune response leading to complications such as severe fever and cerebral edema (4, 5).

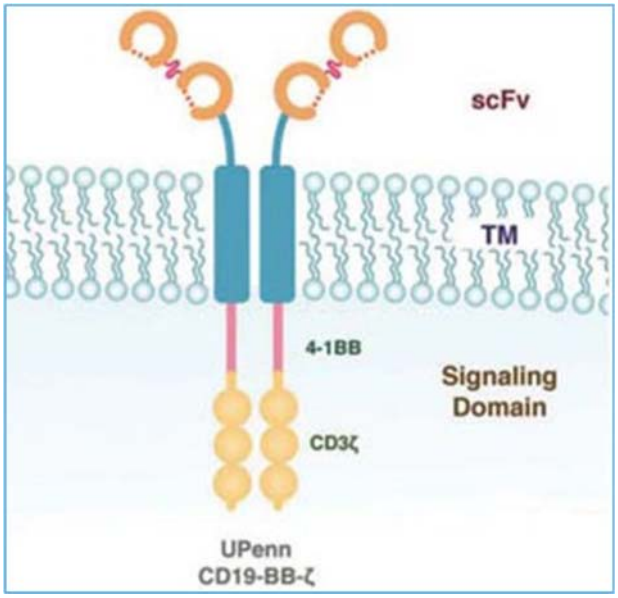

Figure 1. *Schematic diagram of the CAR-T receptor. Tisagenlecleucel possesses a CD137 (4-1BB) costimulatory domain (pink), a signalling CD3-zeta domain (pale orange) and a murine scFv (dark orange) in addition to spacer and transmembrane domains (light blue). Modified from (Leahy et al., 2018).*

3.1.2 Clofarabine

3.1.2.1 Pharmacology

Clofarabine is a second-generation purine nucleoside analog, designed for higher efficacy and reduced extramedullary toxicity compared to fludarabine and cladribine (Jeha et al., 2023). It is toxic to both non-dividing and rapidly proliferating lymphocytes. Clofarabine resists phosphorylation cleavage and remains stable in acidic environments (7).

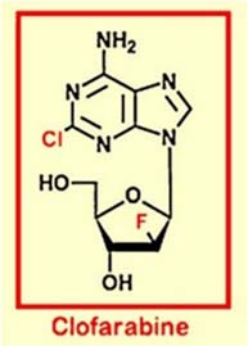

Figure 2. *Molecular structure of Clofarabine: 2-chloro-2'-arabino-fluoro-2'-desoxyadenosine. Modified from (7)*

Approved by the FDA in 2004 (Clolar™) and the European Commission in 2006 (Evoltra®), clofarabine is actively investigated for other cancers and age groups. It is primarily used for pediatric ALL patients with relapsed or refractory disease after at least two prior regimens (Agencia Española de Medicamentos y Productos Sanitarios, 2023).

3.1.2.2 Mechanism of Action

Clofarabine is progressively phosphorylated by deoxycytidine kinase (dCK), monophosphate kinase, and diphosphate kinase to its active form, clofarabine triphosphate (clofarabineTP), which acts via three mechanisms (6-8):

1. Inhibition of DNA polymerase (α and ε): Competes with dATP, halting DNA synthesis and repair.
2. Ribonucleotide reductase inhibition: Depletes dNTP pools.
3. Mitochondrial membrane disruption: Induces apoptosis, including in non-proliferating lymphocytes.

3.1.2.3 Pharmacokinetics

Pharmacokinetics vary with weight. An intravenous infusion of 52 mg/m² daily for five consecutive days provides similar exposure across weights. Dosage is calculated based on actual body surface area. Treatment cycles repeat every 2–6 weeks, depending on hematopoietic recovery (RAN ≥0.75 × 10⁹/L) and baseline organ function. Dose reduction by 25% may be required for significant toxicity (8).

3.1.2.4 Advantages

- Effective for ALL patients.
- Can be combined with other treatments.
- Fewer severe effects compared to Kymriah® (Ramiz et al., 2023).

3.1.2.5 Disadvantages

- High toxicity with increased risk of severe adverse effects.
- Treatment-related infections.
- Severe sepsis with potential mortality (Ramiz et al., 2023).

3.2. Discussions

In this study, treatments based on Tisagenlecleucel and Clofarabine for ALL were contrasted, considering aspects such as mechanism of action, efficacy, advantages, disadvantages, and their clinical impact across diverse subgroups of patients.

Both treatments represent significant advances in the management of ALL, although they have different applications and toxicity profiles. Tisagenlecleucel has been identified as a more innovative option, but it is associated with a higher financial cost. Conversely, clofarabine has been identified as a viable alternative for cases that have relapsed. The development of accessible therapies that carry a reduced risk of complications remains a significant objective in the treatment of ALL.

4. CONCLUSIONS

Choosing between treatments depends on leukemia type, patient age, health status, and previous treatment response. Kymriah® is effective for B-cell ALL, while clofarabine is used for both ALL and acute myeloid leukemia. Regarding age, Kymriah® is suitable for pediatric and adult patients, while clofarabine is more commonly used in pediatric patients. Access to genetic treatments like Kymriah® is currently limited due to high costs compared to non-genetic alternatives. Finally, clofarabine is often employed in relapsed cases or when CAR-T therapy is not an option.

5. DECLARATIONS

5.1. Study Limitations

This comparative review has several limitations that should be acknowledged. The literature search was restricted to two databases (PubMed and Scielo) and publications from 2009-2023, potentially excluding relevant studies from other sources or time periods. As an academic coursework project, the scope and depth of analysis were constrained by institutional requirements regarding length and citation limits. The absence of direct head-to-head clinical trials comparing tisagenlecleucel and clofarabine necessitated indirect comparisons based on separate studies with potentially different patient populations and methodologies. Additionally, the rapidly evolving nature of CAR-T cell therapy means newer data may have emerged since the completion of this analysis. Cost-effectiveness comparisons were limited due to variable pricing across healthcare systems and the lack of comprehensive economic analyses in the available literature.

5.2. Acknowledgements

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5.4. Competing Interests

The authors declare no financial, professional, or personal conflicts of interest that could have influenced the content or conclusions of this review. All authors are affiliated solely with academic institutions and have no commercial relationships with pharmaceutical companies manufacturing the treatments discussed in this analysis.

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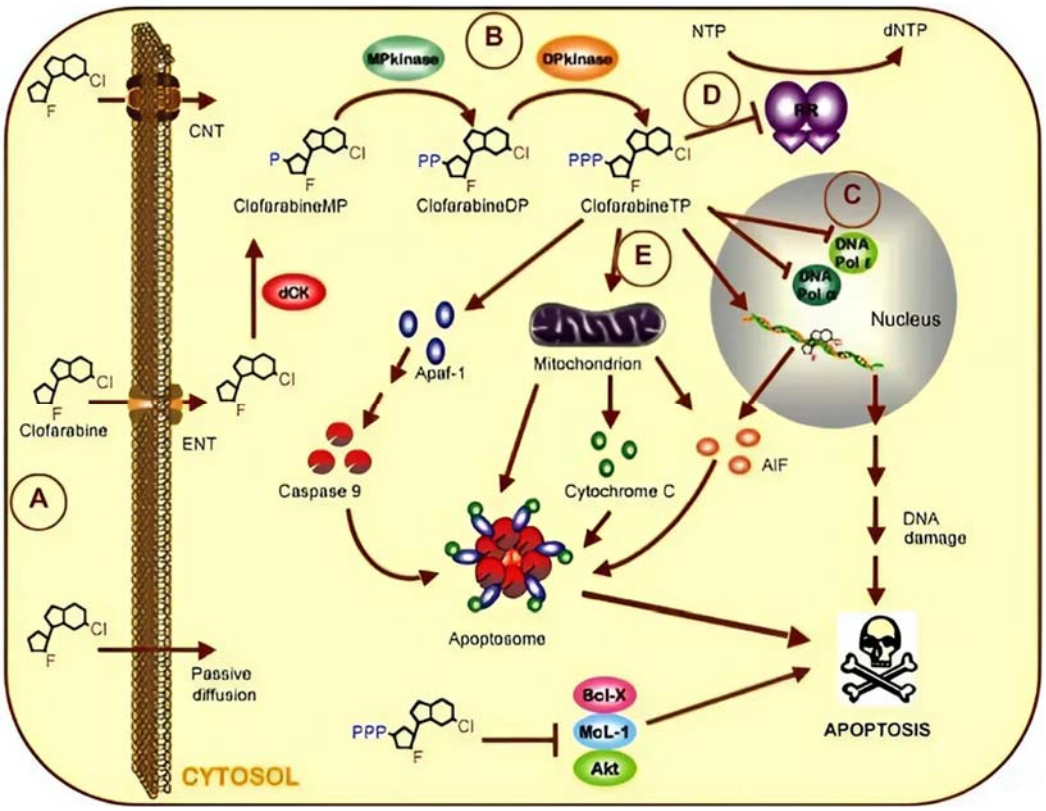

Figure 3. *Mechanism of action of Clofarabine. (A) Entrance of Clofarabine to the cell through three possible pathways: active/facilitated transport by nucleoside transporters or passive diffuison. (B) Progressive phosphorylation by dCK, MPkinase, and DPkinase. (C) Inhibition of DNA polymerase (α and ε). (D) Ribonucleotide reductase inhibition. (E) Mitochondrial membrane disruption. Modified from (Zhenchuk et al., 2009).*

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Interview

FROM AIR FORCE TO HYPERSONIC FUTURE: ÉLCIO GERÔNIMO DE OLIVEIRA'S JOURNEY IN BRAZILIAN AEROSPACE DEVELOPMENT (ENGLISH VERSION)

DA FORÇA AÉREA AO FUTURO HIPERSÔNICO: A TRAJETÓRIA DE ÉLCIO GERÔNIMO DE OLIVEIRA NO DESENVOLVIMENTO AEROESPACIAL BRASILEIRO (VERSÃO EM INGLÊS)

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The complete version of the interview is available at: https://youtu.be/FVvgNJ3ujlc?si=y4eSf-hXbZ08wfSF

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ABSTRACT

Introduction: The interview with Élcio Gerônimo de Oliveira, conducted by reporter Luis, presents the professional trajectory of a Brazilian researcher with experience in the Brazilian Air Force and academia, focusing on space systems and hypersonic vehicles. **Objectives:** To document Élcio's career and contributions to Brazilian aerospace development, highlighting his transition from military to academic career and his participation in strategic projects, especially the 14X project. **Methods:** The interview was structured in thematic blocks, addressing the military career, academic experience, and, in greater detail, involvement in the 14X hypersonic vehicle project. Open-ended questions were asked, allowing the interviewee to share his experience and technical knowledge. **Results:** Élcio described his progression in the Brazilian Air Force, from researcher to vice-head of the Space Directorate, highlighting the development of launch vehicles, inertial navigation systems, and the SARA project. He reported his transition to an academic career, including his experience as a professor at Luleå University of Technology in Sweden. Élcio detailed his coordination in the 14X project, a hypersonic vehicle that reached Mach 7, with prospects of reaching Mach 10. **Discussion:** The interview reveals the importance of international cooperation and technology transfer, exemplified by the donation of Brazil's first hypersonic laboratory. It also highlights the technical challenges in building hypersonic vehicles and the potential of these technologies for military and civilian applications. **Conclusion:** Élcio Gerônimo de Oliveira's career exemplifies the Brazilian contribution to advanced aerospace research, demonstrating the national capacity to develop strategic technologies such as hypersonic vehicles, despite resource limitations, and pointing to future possibilities for transportation and space exploration.

Keywords: *Hypersonic, Aerospace, 14X, Propulsion, Rockets..*

RESUMO

Introdução: A entrevista com Élcio Gerônimo de Oliveira, conduzida pelo repórter Luis, apresenta a trajetória profissional de um pesquisador brasileiro com experiência na Força Aérea Brasileira e no meio acadêmico, com foco em sistemas espaciais e veículos hipersônicos. **Objetivos:** Documentar a carreira e contribuições de Élcio para o desenvolvimento aeroespacial brasileiro, destacando sua transição da carreira militar para a acadêmica e sua participação em projetos estratégicos, especialmente o projeto 14X. **Métodos:** A entrevista foi estruturada em blocos temáticos, abordando a carreira militar, a experiência acadêmica e, com maior detalhamento, o envolvimento no projeto do veículo hipersônico 14X. Foram realizadas perguntas abertas, permitindo ao entrevistado compartilhar sua experiência e conhecimento técnico. **Resultados:** Élcio descreveu sua progressão na Força Aérea Brasileira, desde pesquisador até vice-chefe da Diretoria de Espaço, destacando o desenvolvimento de veículos lançadores, sistemas de navegação inercial e o projeto SARA.

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Relatou sua transição para a carreira acadêmica, incluindo sua experiência como professor na Universidade de Luleå, na Suécia. Élcio detalhou sua coordenação no projeto 14X, um veículo hipersônico que alcançou Mach 7, com perspectivas de atingir Mach 10. **Discussão:** A entrevista revela a importância da cooperação internacional e da transferência de tecnologia, exemplificada pela doação do primeiro laboratório de hipersônica do Brasil. Evidencia também os desafios técnicos na construção de veículos hipersônicos e o potencial dessas tecnologias para aplicações militares e civis. **Conclusão:** A carreira de Élcio Gerônimo de Oliveira exemplifica a contribuição brasileira para pesquisa aeroespacial avançada, demonstrando a capacidade nacional de desenvolver tecnologias estratégicas como veículos hipersônicos, apesar das limitações de recursos, e apontando possibilidades futuras para o transporte e exploração espacial.

Palavras-chave: *Hipersônica, Aeroespacial, 14X, Propulsão, Foguetes.*

1. INTRODUCTION

In an era of rapid technological advancement and space exploration, Brazil has stood out with significant contributions in the aerospace field. In this exclusive interview, we speak with Élcio Gerônimo de Oliveira, a central figure in the development of Brazilian strategic technologies. With a career that spans from the Brazilian Air Force to international academia, Élcio shares his experience in developing space systems and, particularly, his contribution to the 14X project, a Brazilian hypersonic vehicle that represents a milestone in national aerospace engineering. His trajectory not only illustrates Brazilian scientific potential but also reveals the challenges and achievements of someone who dedicates their professional life to technological innovation in a sector vital to the country's sovereignty and development.

Luis: Good afternoon, Élcio Gerônimo de Oliveira. How are you, sir?

Élcio: Good afternoon, Mr. Luis de Boni. How are you? Everything well?

Luis: Thank you very much for receiving us today. Our interview will be published in Portuguese by Periódico Tchê Química, in English by the Southern Journal of Sciences, and we will share this interview with a local television station, Conecta Mais TV. The interview content will be shared under a Creative Commons license. Is this acceptable to you, sir?

Élcio: No problem.

Luis: By the way, would you prefer that I address you as professor or doctor?

Image 1: Dr. Élcio Gerônimo de Oliveira.

Élcio: No, no formality is needed, but Élcio is sufficient.

Luis: Thank you very much, Élcio. I'll start by asking questions about your career. Are you agreeing?

Élcio: Agreed.

Luis: First question. You held several positions in the Brazilian Air Force, such as research officer, head of the space systems division, and deputy director of the space subdirectorate. Could you describe your most significant achievements and challenges in these roles?

Élcio: It was indeed a very dynamic career. I started in 2007 as a researcher in this area and remained until 2018, when I left the Air Force. During this period, I initially worked as a

researcher in the area of flight dynamics. Later, I worked as a researcher in the space vehicle control area, specifically rocket control. Then, I worked in the flight dynamics area, where I was already serving as deputy head of the sector, and in my career progression I became head of the space systems division, which included within the division the aerodynamics sector, structure, control, flight dynamics, and the project itself, all the rocket design part.

That was the composition, and later I assumed the role of deputy chief of the Space Directorate of the General Space Institute, where we had ten subordinate divisions. So, I went from heading one division to being deputy chief of the directorate, which encompassed ten divisions, including my former space systems division, plus the chemistry division, mechanics division, electronics division, and testing division. These ten divisions comprised what we could call the industrial and research park for the Air Force's rocket area. It was where we developed and manufactured the components used in building our rockets, from the propulsion part, the electronics part, metal structures, carbon fiber structures, and, in short, all the necessary elements that were available in our directorate.

Luis: This is located in São Paulo?

Élcio: Yes, it's in São Paulo, more precisely in São José dos Campos.

It's difficult to enumerate what the most important points were within the career because we dealt with various elements throughout this period, but the development of launch vehicles was particularly noteworthy. I was involved in the old VLS project, later in the VLM project, in developing the engine for this rocket, the microsatellite launch vehicle. I also participated as project manager for the atmospheric reentry satellite, SARA, intended for research, and the navigation and control system for rockets that we developed internally. This last one represents a strategic base product, the inertial navigation system, which is a restricted item that is not easily available on the market, as it involves sensitive or embargoed technology that is difficult to access. We developed and produced it, and my doctorate and a significant part of my time at the Institute were dedicated to developing this inertial navigation system. I consider these three points to be the most important in my career, in terms of work, although we had the opportunity to deal with all imaginable types of activities, including meteorological studies, making this period very interesting and dynamic.

It was an excellent career in the Air Force. It was quite intensive, requiring many hours of planning and study, reviewing literature and theory, because it's necessary to constantly adapt, as there are always new challenges. You need to continuously update and prepare yourself to answer questions and solve problems that occur during project development. Since no project is perfect and problems always arise, it's necessary to find solutions, frequently turning to books, research, travel, getting to know other institutions, talking with specialists in the area, and taking courses, which made this period quite intense in this aspect.

Luis: Thank you very much. Let's move to our second question, just noting that I'm not a professional reporter, but considering the circumstances, I appreciate your patience.

Élcio: No problem, we can proceed.

Luis: Élcio, you made the transition from the military environment to academia, working as a professor and researcher at universities, such as Luleå University of Technology in Sweden - sorry for the possible incorrect pronunciation. What motivated this career change, and how did you adapt to the academic environment?

Élcio: There's no need to apologize, because it really is a difficult name to pronounce. Normally in Portuguese, it would be pronounced "Luleia," but since the A has a circle above it, the pronunciation ends with U, so it's "Luliu" - that's the correct pronunciation of the name.

Actually, it was a very smooth transition because during the period I was working at IAE, at the Institute, I had completed my doctorate at ITA and was invited by ITA to be a professor in one of the disciplines that was part of my training, related to Kalman filters, dynamic systems optimization, and Kalman filtering.

This period provided me with a lot of experience because master's and doctoral courses are offered at ITA. I had students under my guidance as well, and this invitation, this work at ITA, was something that motivated me a lot and made me comfortable with the academic environment. I also wanted to do this for an internal feeling of giving back. I taught at ITA without remuneration, as a guest professor,

offering my participation for free, without receiving payment for it, and I considered this a counterpart for the years I studied there during my doctorate. I did four years of doctorate at ITA and then worked for approximately four to five years as a guest professor, which constituted this counterpart and gave me a lot of experience. I taught from 2012 to 2017 at ITA, totaling five years, which gave me a very solid foundation from the point of view of academic coexistence, scientific development, guidance, and other activities.

When I ended my period in the Air Force, which is a normal process upon completing the time of service, I requested my reserve status, already having a good relationship with some institutions and international researchers. At a certain point, I received an invitation to spend a year at Luleå University as a visiting researcher and professor in the rocket area, as they were interested in developing this area. I accepted the invitation to spend a year, later participated in a competition for a permanent professor position at the university, was approved, took the position, and remained three years in Sweden, living and working as a permanent professor.

During this period, the pandemic occurred, and some difficulties began, such as the impossibility of traveling and accompanying my family in Brazil. I had a mother of a certain age, who began to create situations that made my return to Brazil difficult. So, at the end of the third year, I decided to leave the university and return definitively to Brazil to be with my family and offer support.

The transition was very smooth; the academic structure there is excellent, and the university is very well organized. They significantly value pedagogical and didactic issues, which was very beneficial. In my training as a physicist, I also completed all the academic parts related to didactics, teaching psychology, and teacher certification, and one of my goals was, at a certain point in life, to assume the role of teacher, which would be quite useful in the activity.

It was very easy; the didactic part was very smooth, the classes were very similar to what I already taught here in Brazil, and I began to guide students following the same profile and the same line we adopted here, without major problems. I believe the biggest difficulty was dealing with the students' accents. I had a class with 40 students, approximately 10 to 15 Swedes, about 15 Indians, and various Europeans from different parts of Europe. When they all gathered in a room, it was interesting because each one spoke differently, with distinct accents, and with mutual comprehension difficulties. It took about a month to adapt and understand what each one was saying.

Luis: Supposedly, they all spoke English.

Élcio: Everyone spoke English, however, I had a British student in the room, a student from Latvia, a Spaniard, an Italian, an Indian, a Mexican, and you can imagine the diversity of accents. In the first days, it was quite challenging until I could understand. I consider this to be the most challenging and interesting part because there were moments when communication was really difficult due to very pronounced accents. In certain situations, I needed to interrupt and ask them to repeat, because although we all spoke English, we are not native speakers - I am not native - so getting used to this multicultural environment was a challenge, as well as dealing with cultural issues, considering that each individual has a different culture, from personal mentality influenced by their country's culture to religious issues. I believe this was the biggest difficulty: dealing with this cultural and origin diversity at the university. The rest was smooth; the technical part didn't present major problems.

Luis: Very interesting. Professor Élcio, we'll now move to a second block of questions related to your professional activities. As flight test coordinator of the 14X project at the Institute for Advanced Studies, IAV, what were your main responsibilities, and what was the most challenging aspect of this role?

Élcio: This is an interesting question. Remember I mentioned that I had left the Air Force and later went to Luleå University? Actually, there was a significant 8-month interval. When I left the Air Force, in the last year as deputy chief of the Space Directorate, my function was to be the point of contact between the group that manufactured the rocket and the group that was developing the 14X project, which is a hypersonic vehicle.

Imagem 2: Visualização do projeto 14-X.
Image source: provided by the author (Dr. Oliveira)

They were developing an engine and wanted to test it in flight. Since I had worked in the control area, flight dynamics, and had been head of various sectors, I had a comprehensive view of the vehicle, the rockets, and how to use them to conduct this test. So, my last year in the Air Force was dedicated to helping my colleagues at IAV, at the Institute, in project decision-making and studies, in developing studies, mainly those related to the trajectory to position the engine in the ideal condition for its operation.

The hypersonic engine has certain particularities. It only operates from the moment it reaches a certain speed, a certain Mach number, which already represents hyperspeed, and at a certain altitude, defined by the project, where it encounters a certain air density. Therefore, it combines hyperspeed with a certain air density, entering the favorable regime for operation, at which point the engine starts to function and accelerates the vehicle to an even higher speed, continuing to advance.

Luis: Allow me to ask some additional questions on your topic.

Élcio: Certainly, feel free.

Luis: First, I followed the 14-X through news reports, I didn't know you were involved in this project. My first question is: Who assigned the name to the project, if you know?

Élcio: I believe, if I'm not mistaken, it was the researcher considered the father of the project in Brazil's propulsion area, Colonel Salamoni. He assigned the name 14-X in honor of Santos Dumont, who created the 14-Bis. It was his choice, along with his team at the time. If I'm not wrong, that's the story, although some details may be lost over time. In an informal conversation I had, I curiously asked about the origin of the name, and they explained the reference to 14. Therefore, the 14 refers to the 14-Bis, and the X designates the hypersonic project, adding an element that alludes to a secret project.

Imagem 3: Caça A1
Image source: Chris Lofting.
https://pt.wikipedia.org/wiki/AMX_A-1#/media/Ficheiro:FAB_AMX_International_A-1A_-_Lofting.jpg.

Normally, we see this nomenclature in other projects, like the A1 Fighter project, originally called AMX, which was developed in partnership between Brazil and Italy. The 14X follows this logic: when it's a test or prototype, the X is used. In fact, the flight test institute has the X as its symbol. This is already part of the institutional culture. And the 14 originated from the 14-Bis.

Luis: Returning to the main question, I appreciate the information, which was very interesting and allowed the audience to learn a bit of these stories. It's also important to recognize these people who were true pioneers, often unknown to the general public.

Élcio: To illustrate the importance of this colonel, he received the first hypersonics study laboratory in Brazil from the Air Force as a donation from his hypersonics advisor in the United States. He completed his doctorate there, and his advisor was the owner of the laboratory. When the advisor retired, he donated the laboratory to him. The laboratory was then transferred to the Air Force and installed there, becoming the country's first hypersonics laboratory. This demonstrates the importance of international relations and research. Imagine the difficulty of setting up a hypersonics laboratory from scratch at that time.

Luis: It would be practically impossible.

Élcio: Considering the necessary resources. This is a historical fact. The laboratory

even received the name of this professor, Professor Nagamatsu, in honor of this American academic who made the donation. The story behind this project is truly remarkable and significant.

When I left the Air Force, I was thinking of dedicating myself to the academic area. However, the team felt my absence, commenting that they had lost the person who made the connection between the projects. So they invited me to work temporarily on the project. It was an external arrangement, since I was outside the Air Force, being hired as a kind of consultant to set up this project, the flight test project. There's the rocket project, the hypersonic engine project, and to unite these elements, a specific project is needed: the flight test project, which is the flight test project.

So, I defined the requirements. I had an excellent team. I talked with the hypersonics area specialists, who informed me of their needs, and with my team, who informed me of what was possible to accomplish. Occasionally, conflicts arose, which I mediated, aligning everything so we could reach viable solutions.

When I left the Institute, the flight test project was already completed. I left the Institute to go to Sweden, to Luleå. In 2019, I published a scientific article about this 14X test. I left at the beginning of 2019, more precisely at the end of 2018. At the beginning of 2019, I went to Sweden, and in that same year, I published the article about the 14X test, which is available online. The flight was scheduled for 2020, if I'm not mistaken.

Luis: This is my next question. Understand that I follow from afar, not in person, but through the internet. Did the launch occur successfully?

Élcio: Yes, it occurred successfully. We adapted the hypersonic engine, which actually has two engines. It was quite interesting because the hypersonic engine has a particular configuration. Imagine a plane with a bevel, which is the intake. Similar to American fighter aircraft that have square turbines, with square air intakes. The design is similar: a plane with a square intake, containing internally all the processing necessary to generate thrust, hypersonic propulsion.

Two of these engines were used positioned frontally, creating a quite peculiar configuration. These engines were coupled to the end of a rocket. The intakes, these bevels, were positioned at the tip, giving it a shape resembling a screwdriver. The complete rocket, observing its end, resembled a screwdriver. The interesting thing is that, instead of the traditional conical tip, it ended with a straight line.

It was an interesting challenge to reach the final configuration and conduct the necessary aerodynamic studies to make the flight viable. The test was carried out, the vehicle reached the necessary flight conditions, and the engine was activated and functioned adequately. Currently, they're already in the preparation phase for a second test, which will involve more rigorous conditions, aiming to reach another level in the project requirements.

The launch occurred in 2021, when I was no longer in Brazil, or perhaps in 2020; I don't remember precisely, it would be necessary to verify. It was very gratifying because it was a project that I had outlined and that was delivered ready to function. I didn't conduct the study alone, I just coordinated, but we had an exceptional team that conducted all this work, both from IAE and IAV, very competent teams that provided support for this to be realized. These are distinct phases of development.

Luis: Do you believe it would be possible to manufacture the 14-X at scale and find other applications?

Élcio: The 14X is...

Luis: Or what products could derive from it.

Élcio: We conducted the engine test, and the next test will be of the complete vehicle. The vehicle resembles a board with these two engines coupled to the lower part, configuring a small aircraft. It presents a Delta-type format, an interesting aerodynamic configuration, containing all the requirements necessary for this design, to meet the flight requirements. It uses the same engines that have already been tested. However, this aircraft needs to be accelerated by a rocket or some other means to reach the conditions necessary for its autonomous operation.

For understanding, the 14-X flight, more specifically the 14-XS, which refers to the engine, needed a rocket to accelerate to approximately Mach 7. At this speed, the engine starts to function.

Luis: To make a comparison, in the movie Top Gun 2, the aircraft reached Mach 10.

Élcio: Exactly. The next step, with the complete aircraft, has the nominal requirement to reach Mach 10. Our company, which provides consulting in this area, has already conducted a study and we managed to develop a rocket configuration capable of reaching Mach 10, positioning any hypersonic vehicle in this condition. Once the vehicle is placed in this condition, it proceeds autonomously.

Luis: Allow me to ask one more question, this being the last one.

Élcio: Certainly.

Luis: When the launch is carried out, does it occur from the ground or does it need to be done from another aircraft?

Élcio: It's possible to carry out both from the ground and from another aircraft. We carried out from the ground, especially for higher Mach speeds. For higher Mach speeds, a heavier and larger rocket is necessary, which represents a significant load. Adapting this to an aircraft is more complex and more challenging, requiring many studies. It's more practical to position it on a rocket and launch from the ground, allowing it to fulfill its mission autonomously.

Within this context, the most challenging aspect for me in the 14-X project was, first, developing the mission project, the test project, and coordinating the two teams, identifying the balance points to position the engine in adequate conditions, respecting the capacities and limitations of the rocket used. It was a very interesting process, involving many studies to reach the final configuration of the project, making it successful.

There are other applications for the hypersonic area that are relevant for space research and transportation. As mentioned, the movie presents an aircraft that reaches Mach 10. Historically, we had the Concorde, an extremely high-speed aircraft, which was deactivated due to operational costs and other issues. However, nothing prevents us from developing a means of transportation based on these engines in the future to carry out some type of mission different from current ones.

Luis: Different from military applications, for civilian purposes.

Élcio: Yes, civilian applications also exist.

Luis: I hope to be able to use this technology someday.

Élcio: Who knows in the future. We'll see.

Luis: I'm an optimist by nature.

Élcio: We share this characteristic.

This interview was part of the interinstitutional scientific dissemination partnership project of the SSCON – 2024 conference, continuing for the future 2026 edition in RJ.

Image: SSCON 2024 Logo.

DECLARATIONS

1. Limitations: The interview is limited to its content.

2. Funding source: The host funded this interview.

3. Conflicts of interest: The host has worked for the journal for many years, and this may have influenced the interview.

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RESEARCH LANDSCAPE OF REPURPOSED MEDICATIONS IN CANCER TREATMENT: A MULTI-DATABASE BIBLIOMETRIC ANALYSIS OF ELEVEN OFF-PATENT THERAPEUTICS

PANORAMA DE PESQUISA DE MEDICAMENTOS REPOSICIONADOS NO TRATAMENTO DE CÂNCER: UMA ANÁLISE BIBLIOMÉTRICA MULTI-BASE DE DADOS DE ONZE TERAPÊUTICOS FORA DE PATENTE

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ABSTRACT

Background: Drug repurposing offers potential advantages for cancer therapy development, particularly when utilizing medications with established safety profiles and expired patents. While individual repurposed medications have been investigated for oncological applications, comprehensive comparative analyses of research distribution patterns across multiple therapeutic candidates appear limited in the literature. Understanding these patterns may provide insights into research priorities and potential knowledge gaps. **Aim**: This exploratory study was designed to quantify and compare the volume of scientific literature examining the anticancer potential of eleven selected off-patent medications across different pharmacological classes. **Methods**: Bibliometric searches were conducted across five databases (Google Scholar, BVS, PubMed, NIH, and Science.gov) using standardized search terms combining each medication name with "cancer" and "cancer treatment." The selected medications included ivermectin, fenbendazole, mebendazole, albendazole, metformin, propranolol, disulfiram, valproic acid, thalidomide, dexamethasone, and hydroxychloroquine. Basic statistical analyses were performed to examine the distribution patterns and correlations within the database. **Results**: The search yielded 3,226,066 total publications with considerable variation in distribution patterns. Dexamethasone accounted for the largest proportion (1,538,058 publications, 47.68%), followed by metformin (697,172 publications, 21.61%). Some medications with smaller overall publication volumes demonstrated higher proportions of treatment-specific research, such as fenbendazole (87.82%), disulfiram with copper (86.54%), and hydroxychloroquine with zinc (75.21%). The Herfindahl Index indicated a high concentration of research attention (0.2870). **Discussion**: The findings suggest substantial variation in research attention across the selected medications. While some medications dominate the literature, others with focused treatment-specific research may warrant further investigation. The inverse relationship observed between total publication volume and treatment specificity suggests that research patterns in this field may be more complex than absolute publication counts indicate. **Conclusions**: This preliminary bibliometric assessment reveals an uneven distribution of research attention among repurposed medications being investigated for cancer applications. These patterns may inform future research prioritization, though further qualitative analysis would be valuable to assess the clinical significance of these quantitative observations.

Keywords: *Drug repositioning, off-patent pharmaceuticals, oncology research patterns, pharmacological classes, research concentration analysis.*

RESUMO

Introdução: O reposicionamento de medicamentos oferece vantagens potenciais para o desenvolvimento de terapias contra o câncer, particularmente ao utilizar medicamentos com perfis de segurança estabelecidos e patentes expiradas. Embora medicamentos reposicionados individuais tenham sido investigados para aplicações oncológicas, análises comparativas abrangentes dos padrões de distribuição de pesquisa entre múltiplos candidatos terapêuticos parecem limitadas na literatura. Compreender esses padrões pode fornecer insights sobre prioridades de pesquisa e potenciais lacunas de conhecimento. **Objetivo:** Este estudo exploratório foi

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desenvolvido para quantificar e comparar o volume da literatura científica que examina o potencial anticancerígeno de onze medicamentos selecionados fora de patente, pertencentes a diferentes classes farmacológicas. **Métodos:** Pesquisas bibliométricas foram conduzidas em cinco bases de dados (Google Scholar, BVS, PubMed, NIH e Science.gov) utilizando termos de busca padronizados combinando cada nome de medicamento com "câncer" e "tratamento de câncer". Os medicamentos selecionados incluíram ivermectina, fenbendazol, mebendazol, albendazol, metformina, propranolol, dissulfiram, ácido valproico, talidomida, dexametasona e hidroxicloroquina. Análises estatísticas básicas foram realizadas para examinar os padrões de distribuição e correlações dentro da base de dados. **Resultados:** A busca resultou em 3.226.066 publicações totais com variação considerável nos padrões de distribuição. A dexametasona representou a maior proporção (1.538.058 publicações, 47,68%), seguida pela metformina (697.172 publicações, 21,61%). Alguns medicamentos com volumes menores de publicação total demonstraram proporções mais altas de pesquisa específica para tratamento, como fenbendazol (87,82%), dissulfiram com cobre (86,54%) e hidroxicloroquina com zinco (75,21%). O Índice de Herfindahl indicou uma alta concentração de atenção de pesquisa (0,2870). **Discussão:** Os achados sugerem variação substancial na atenção de pesquisa entre os medicamentos selecionados. Embora alguns medicamentos dominem a literatura, outros com pesquisa específica focada em tratamento podem merecer investigação adicional. A relação inversa observada entre o volume total de publicações e a especificidade do tratamento sugere que os padrões de pesquisa neste campo podem ser mais complexos do que os números absolutos de publicações indicam. **Conclusões:** Esta avaliação bibliométrica preliminar revela uma distribuição desigual da atenção de pesquisa entre medicamentos reposicionados sendo investigados para aplicações em câncer. Esses padrões podem informar futuras priorizações de pesquisa, embora análises qualitativas adicionais sejam valiosas para avaliar a significância clínica dessas observações quantitativas.

Palavras-chave: *Reposicionamento de medicamentos, farmacêuticos fora de patente, padrões de pesquisa oncológica, classes farmacológicas, análise de concentração de pesquisa.*

1. INTRODUCTION

This study presents a quantitative bibliometric analysis of research volume in the scientific literature regarding the potential use of 11 medications not protected by patents in the treatment of different types of cancer. The medications analyzed in this bibliometric study are: Ivermectin, Fenbendazole, Mebendazole, Albendazole, Metformin, Propranolol, Disulfiram, Valproic Acid, Thalidomide, Dexamethasone and Hydroxychloroquine.

The primary objective of this research was to quantify and compare the volume of scientific literature examining the anticancer potential of these drugs, providing a numerical perspective on research distribution patterns and the allocation of scientific attention across different pharmacological classes.

Result quantification was performed through bibliometric searches across five databases, including:

- Google Scholar: https://scholar.google.com/
- BVS (Virtual Health Library): https://bvsalud.org/
- PubMed: https://pubmed.ncbi.nlm.nih.gov/
- NIH (National Institutes of Health): https://www.nih.gov/
- Science.gov: https://www.science.gov/

The counting methodology recorded total search results without categorizing studies by type (*in vitro*, *in vivo*, clinical trials), by the proposed mechanism of action, or by type of cancer investigated. This quantitative bibliometric approach enabled the identification of patterns and trends in research attention distribution, highlighting which medications present the greatest volume of research interest for their repositioning in cancer treatment.

This bibliometric analysis aims to provide an initial overview of research distribution patterns for these off-patent drugs, thereby informing future research priorities and potentially guiding the investigation of accessible therapeutic alternatives for cancer treatment.

1.1. Literature Review

1.1.1. IVERMECTIN

Ivermectin was originally developed as a broad-spectrum antiparasitic medication. It was discovered in the late 1970s by researchers Satoshi Ōmura and William Campbell, who subsequently received the Nobel Prize in

Physiology or Medicine in 2015 for this discovery. As documented by Crump and Ōmura (2011), ivermectin is a derivative of avermectin, a substance isolated from a Streptomyces bacterium found in a soil sample collected near a golf course in Japan. This discovery revolutionized the treatment of parasitic infections, being particularly effective against onchocerciasis (river blindness) and lymphatic filariasis (Crump & Ōmura, 2011, p. 13-28).

It was introduced to the veterinary market in 1981 under the trade name Ivomec, initially used to control parasitic infections in livestock and companion animals. In 1987, it was approved for human use for the first time when Merck initiated the Mectizan® Donation Program for treating onchocerciasis in endemic countries. According to Crump and Ōmura (2011), "the use of ivermectin in humans began in 1987 when Merck donated the medication to treat onchocerciasis in developing countries" (p. 17). FDA approval for human use was granted in 1996 for the treatment of strongyloidiasis and onchocerciasis (Campbell et al., 2012, pp. 853-865).

The side effects of ivermectin are generally mild and transient when used at recommended doses. According to Kaur *et al*. (2021), the most common adverse effects include "transient skin reactions, pruritus, fever, nausea, diarrhea, dizziness, arthralgia, and myalgia" (p. 1342). In patients with high parasite loads, the Mazzotti reaction may occur, characterized by fever, headache, pruritus, lymphadenitis, and edema. At elevated doses, more serious neurotoxic effects may occur, though these are rare. Santin *et al*. (2021) observe that "ivermectin has an excellent safety profile, with more than 2.5 billion doses distributed globally over nearly four decades" (Santin *et al*., 2021).

The original patent for ivermectin, granted to Merck & Co., expired in the late 1990”s (Crump & Ōmura, 2011, p. 25). The exact expiration date varies by source (1996-1998) due to patent extensions and different jurisdictions. The USPTO documents show the original patent was scheduled to expire in 1997 but was extended multiple times (U.S. Patent and Trademark Office, 1998). Since then, various generic formulations have become available in the global market, resulting in greater accessibility and lower medication costs. Currently, ivermectin is available as a generic medication and is widely used in public health programs across various regions worldwide. Taylor and Greene (1989), in their pioneering study on ivermectin use for treating human onchocerciasis, already highlighted this medication's potential for public health programs due to its efficacy and safety—characteristics that subsequently enabled its widespread use in resource-limited regions following the expiration of its patent.

Beyond its traditional antiparasitic applications, ivermectin has been investigated for various other conditions. Tang *et al*. (2021) document that "ivermectin possesses powerful antitumor effects, including inhibition of proliferation, metastasis, and angiogenic activity in a variety of cancer cells." Recent studies have also investigated its potential as an antiviral agent against various viruses, including dengue, influenza, and SARS-CoV-2. Caly *et al*. (2020) demonstrated *in vitro antiviral activity against SARS-CoV-2; however,* subsequent randomized clinical studies have not confirmed significant efficacy in COVID-19 patients (Popp *et al*., 2021). In dermatology, it is used to treat scabies, rosacea, and pediculosis (Kaur *et al*., 2021).

1.1.2. FENBENDAZOLE

Fenbendazole, developed in the 1970s, represents a milestone in the history of broad-spectrum veterinary anthelmintics. Belonging to the benzimidazole family, this molecule is part of a group that revolutionized the treatment of parasitic infections in animals (McKellar & Scott, 1990). Fenbendazole demonstrates efficacy against a wide range of nematodes (roundworms) and cestodes (tapeworms) in various animal species (McKellar & Scott, 1990). Its mechanism of action involves binding to β-tubulin, inhibiting microtubule polymerization in parasites, and blocking glucose absorption, leading to parasite death through energy depletion (Dogra *et al*., 2018). Pharmacokinetic studies in sheep have demonstrated properties that contribute to its therapeutic profile (Marriner & Bogan, 1981). The selectivity of fenbendazole for parasites over host cells contributes to its excellent safety profile, establishing it as a crucial medication for controlling gastrointestinal parasitic infections (McKellar & Scott, 1990).

Fenbendazole was introduced to the veterinary market in 1974 by the pharmaceutical company Hoechst under the trade name Panacur. Marriner and Bogan (1981) noted that fenbendazole was approved for veterinary use in the early 1970s and quickly became a reference anthelmintic for various animal species. Since its introduction, the medication has been widely used

to treat parasitic infections in domestic and production animals (McKellar & Scott, 1990).

Fenbendazole is generally considered safe when used at recommended doses. According to Booze and Oehme (1983), "*fenbendazole demonstrates low acute and chronic toxicity, with a wide safety margin at therapeutic doses*." Adverse effects are rare but may include mild gastrointestinal discomfort, such as decreased appetite and diarrhea. At very high doses, cases of reversible pancytopenia have been reported. As documented by Villar *et al*. (2007), "*in toxicity studies in rats, extremely high doses (1000 mg/kg) for prolonged periods were necessary to induce significant adverse effects*."

The original fenbendazole patent expired decades ago. Patent database records indicate that the original fenbendazole patent expired, allowing generic versions to be produced by various manufacturers. Currently, the medication is produced as a generic by several companies worldwide, available for veterinary use under different trade names and formulations, contributing to its widespread availability and accessibility in the global veterinary products market.

Although fenbendazole is primarily a veterinary medication used to treat parasitic infections, recent studies have investigated its potential anticancer properties. Nguyen *et al*. (2024) documented that "fenbendazole demonstrated anticancer activity against various tumor cell lines, including colorectal cancer, lung cancer, and cells resistant to conventional chemotherapy." Park *et al*. (2022) demonstrated that "fenbendazole was more effective than albendazole against 5-fluorouracil-resistant colorectal cancer cells, inhibiting cell proliferation in a time- and dose-dependent manner." Despite these promising *in vitro* and animal model results, fenbendazole is not approved for human use, and controlled clinical studies are needed to evaluate its safety and efficacy in treating cancer in humans.

1.1.3. MEBENDAZOLE

Mebendazole was originally developed as a broad-spectrum anthelmintic for human use by Janssen Pharmaceutica in Belgium. According to Dayan (2003), mebendazole is a "synthetic benzimidazole developed specifically for treating intestinal nematode infections in humans." It was designed to treat infections caused by Ascaris lumbricoides (ascariasis), Enterobius vermicularis (enterobiasis), Trichuris trichiura (trichuriasis), Ancylostoma duodenale and Necator americanus (hookworm disease), common intestinal parasites in humans (Pawluk *et al*., 2015).

Mebendazole was introduced to the market in 1971 by Janssen Pharmaceutica. According to Braithwaite *et al*. (1982), "*mebendazole was approved for human use in 1971 and introduced to the market under the trade name Vermox*." The medication was approved by the U.S. FDA in 1974 for treating helminthic infections in humans. It quickly became one of the most widely used anthelmintics worldwide, being included in the World Health Organization's List of Essential Medicines (Meco *et al*., 2023).

The side effects of mebendazole are generally mild and transient when used at recommended doses for treating parasitoses. According to Dayan (2003), "the most common adverse effects include abdominal pain, headache, nausea, diarrhea, and occasionally dizziness." In rare cases, especially with prolonged treatments or high doses, transient elevation of hepatic enzymes may occur. As documented by Palmeirim *et al*. (2018), "mebendazole has a good safety profile when administered as a single dose (500 mg), with few reported adverse effects." At very high doses used in clinical trials for cancer treatment, more significant adverse effects have been reported, including myelosuppression.

According to Pawluk *et al*. (2015), "*the mebendazole patent expired in the early 1990s, allowing the production of generic formulations by various manufacturers*." Currently, the medication is produced as a generic by several pharmaceutical companies worldwide, and it is available in various countries as a low-cost treatment for helminthic infections. However, new formulations and specific polymorphs of mebendazole may be protected by more recent patents, such as polymorph C of mebendazole, which has been the subject of new patents for oncological use (Bai *et al*., 2015). Additionally, novel pharmaceutical combinations involving mebendazole continue to be developed and patented, such as synergistic compositions combining mebendazole with nitazoxanide for enhanced antiparasitic spectrum (Fiore, 2015).

Beyond its traditional use as an anthelmintic, recent research has explored mebendazole's potential as an antineoplastic agent. Pantziarka *et al*. (2014) documented that "preclinical studies demonstrated anticancer

activity of mebendazole against various tumor types, including glioblastoma, melanoma, colorectal cancer, and lung cancer." According to Bai *et al*. (2011), "mebendazole showed a significant survival benefit in preclinical models of glioblastoma multiforme." Meco *et al*. (2023) highlight that "mebendazole may be a promising candidate for treating brain tumors due to its ability to cross the blood-brain barrier and its well-established safety profile." Several clinical trials are currently underway to investigate its use in oncology, particularly for brain tumors that are resistant to conventional treatments.

1.1.4. ALBENDAZOL

Albendazole was originally developed as a broad-spectrum anthelmintic for treating intestinal parasitic infections. Its initial indication was for treating infections caused by intestinal nematodes, including ascariasis, hookworm disease, trichuriasis, and enterobiasis, due to its ability to inhibit tubulin polymerization, interfering with glucose uptake by parasites (Horton, 2000). According to Dayan (2003), the mechanism of action of albendazole involves selective binding to parasitic β-tubulin, resulting in cytoskeleton disintegration and ultimately leading to parasite death.

The drug was developed by SmithKline & French Laboratories (now GlaxoSmithKline) and received its first regulatory approval in 1977, being introduced to the market in 1978, initially for veterinary use (Horton, 2000; Lacey, 1990). In 1982, it was approved for human use and rapidly became an essential medication for treating various parasitic infections worldwide (Horton, 2009; Dayan, 2003). In 1983, it was included in the World Health Organization's List of Essential Medicines, recognizing its importance in global public health (Keiser & Utzinger, 2008; Gyapong *et al*., 2005).

Albendazole's side effects are generally mild and transient. According to Horton (2000), the incidence of adverse effects reported in the literature is very low, with gastrointestinal disturbances being most frequent, occurring in slightly more than 1% of cases. Documented adverse effects include abdominal pain, nausea and vomiting, headache (particularly in patients with neurocysticercosis), reversible alopecia in prolonged treatments, and alterations in hepatic enzymes.

In prolonged treatments or higher doses, such as in cases of echinococcosis and neurocysticercosis, Dayan (2003) describes that more serious effects may occur, including hematological changes such as leukopenia and, rarely, pancytopenia. For this reason, regular laboratory monitoring is recommended for patients undergoing long-term treatment.

Keiser and Utzinger (2008) note that, despite these adverse effects, albendazole maintains an excellent safety profile when used as a single dose for treating intestinal helminthiases, justifying its widespread use in mass drug administration programs in endemic areas, where the benefits significantly outweigh the potential risks.

Albendazole no longer has valid patent protection. According to 't Hoen *et al*. (2018), many essential medicines, including albendazole, are no longer protected by active patents, allowing for the production of generic versions. Wirtz *et al*. (2017) note that albendazole is included in the WHO's Essential Medicines List and is available as a generic in various countries, thereby contributing to expanded access, particularly in regions endemic for intestinal parasitic diseases. Pedrique *et al*. (2013) note that despite advances in antiparasitic availability, such as albendazole, access challenges persist in some low-income regions where helminthiasis prevalence is highest.

Beyond its original indications for intestinal parasitic infections, albendazole has been used for various other conditions. According to Pawluk *et al*. (2021), the medication demonstrated efficacy in treating neurocysticercosis (a central nervous system infection caused by the larval form of Taenia solium), hydatidosis (also known as echinococcosis, caused by Echinococcus granulosus), cutaneous larva migrans, microsporidial infections in immunocompromised patients, and giardiasis resistant to other treatments.

Recently, studies have investigated the antineoplastic potential of albendazole. Lim *et al*. (2022) demonstrated that the medication exhibits anti-tumor properties in vitro and in vivo by interfering with tubulin polymerization in cancer cells, thereby inhibiting angiogenesis and inducing apoptosis.

1.1.5. METFORMIN

Metformin was originally developed for the

treatment of type 2 diabetes mellitus and is classified as an oral antihyperglycemic agent, specifically a biguanide. Bailey and Day (2004) highlight its historical origins derived from the plant Galega officinalis, while Rena *et al*. (2017) clarify that metformin acts primarily by reducing hepatic glucose production, decreasing intestinal glucose absorption, and increasing insulin sensitivity through improved peripheral glucose uptake and utilization. Viollet *et al*. (2012) detail the underlying molecular mechanisms, explaining that activation of AMP-activated protein kinase (AMPK) is central to many of metformin's metabolic effects. According to Inzucchi *et al*. (2015), its first formal indication was for glycemic control in non-insulin-dependent diabetic patients, especially those with overweight or obesity, and it was recommended as a first-line treatment in major international guidelines for type 2 diabetes management.

Although the medicinal use of plants containing biguanides (such as Galega officinalis) dates to the Middle Ages, metformin as an isolated substance had an interesting trajectory. According to Bailey (2017), metformin was first synthesized in 1922 by scientists Emil Werner and James Bell, as part of research on guanidines. The same author documents that the medication entered the pharmaceutical market only in 1957, initially in France under the trade name Glucophage, after Jean Sterne recognized its therapeutic potential for diabetes. White (2014) explains that in the United States, FDA approval occurred much later, in 1994, due to concerns related to lactic acidosis observed with another biguanide (phenformin) that had been withdrawn from the market in 1977. Rena *et al*. (2017) emphasize that, despite its long history, the complete molecular mechanisms of metformin continue to be elucidated, which has not prevented it from becoming the most prescribed oral antidiabetic medication worldwide, with more than 120 million users.

Metformin's side effects are well-documented in the medical literature. Sanchez-Rangel and Inzucchi (2017) highlight in their review of the clinical use of metformin in type 2 diabetes that the most common adverse effects are gastrointestinal in nature, including diarrhea, nausea, vomiting, and abdominal discomfort. Sanchez-Rangel and Inzucchi also discuss other relevant effects, such as taste alteration (dysgeusia or metallic taste) and vitamin B12 deficiency associated with prolonged use.

Regarding safety, these same researchers emphasize that lactic acidosis, although the most serious adverse effect, is quite rare and generally occurs in patients with predisposing conditions such as renal, hepatic, or cardiac insufficiency. Sanchez-Rangel and Inzucchi recommend regular monitoring of these at-risk patients and emphasize that for most individuals, metformin is a safe medication when appropriately prescribed.

Metformin no longer has valid patent protection for the original molecule. According to 't Hoen *et al*. (2018), many essential medicines, including metformin, are no longer protected by primary patents, allowing broad access to generic versions in global public health programs. According to the World Health Organization (Persaud *et al*., 2019), metformin is one of the fundamental antidiabetic medications listed in the Essential Medicines List, underscoring its importance in the treatment of type 2 diabetes worldwide. Beall *et al*. (2019) observe that although the original patent expired decades ago, patents for specific extended-release formulations and combinations with other antidiabetics may still be valid in some markets, reflecting the pharmaceutical industry's strategy to extend commercial exclusivity of well-established medications.

Metformin has demonstrated benefits in various conditions beyond diabetes. According to the comprehensive review by Lv and Guo (2020), non-diabetic applications of metformin include polycystic ovary syndrome (PCOS), where it improves insulin resistance, restores menstrual cycles and increases ovulation rates; obesity and weight control, promoting modest weight reduction in patients with and without diabetes; cardioprotective effects, with reduction of cardiovascular events independent of glycemic control; cancer prevention, where epidemiological data suggest reduced risk of various cancer types, especially colorectal, pancreatic, and hepatic; and neurodegenerative diseases, with preliminary studies indicating neuroprotective potential. These expanded applications demonstrate the versatile therapeutic value of this medication originally developed for diabetes treatment.

Recent research has investigated the role of metformin in promoting longevity and healthy aging. Barzilai *et al*. (2016) outlined the TAME (Targeting Aging with Metformin) study, a planned clinical trial to investigate how metformin may positively influence metabolic pathways associated with aging, presenting potential as an intervention to delay the development of multiple age-related diseases.

1.1.6. PROPRANOLOL

Propranolol was developed as the first clinically useful non-selective beta-blocker, with original applications in treating cardiovascular conditions. According to Rubin (2007), in his historical review of major discoveries in pharmacology, propranolol was developed by James Black (later Nobel laureate) and initially indicated for treating angina pectoris, cardiac arrhythmias, and hypertension. The medication works by blocking beta-adrenergic receptors (both β1 and β2), inhibiting the effects of catecholamines (epinephrine and norepinephrine) on the heart and other tissues, thereby reducing heart rate, myocardial contractility, and blood pressure.

Propranolol was synthesized and patented in 1962 by British scientist Sir James W. Black at Imperial Chemical Industries (ICI) laboratories. As documented by Quirke (2006), the medication received approval for medical use in 1964, with clinical trials beginning that same year. It entered the pharmaceutical market in the United Kingdom in 1965 under the trade name Inderal. It received FDA approval for use in the United States in 1967, revolutionizing the treatment of cardiovascular disease and establishing beta-blockers as a fundamental therapeutic class. Propranolol's development is considered a milestone in modern pharmacology, earning Sir James Black the Nobel Prize in Physiology or Medicine in 1988.

Propranolol has a well-characterized side effect profile, primarily related to its non-selective mechanism of action. According to Srinivasan (2019), in his historical review of 50 years of propranolol, adverse effects commonly associated with this medication include cardiovascular effects, such as bradycardia, hypotension, and cold extremities due to peripheral vasoconstriction; central nervous system effects, manifested by fatigue, dizziness, and sleep disturbances; respiratory effects, with risk of bronchoconstriction, especially in patients with a history of asthma or COPD; metabolic effects, such as masking adrenergic signs of hypoglycemia in diabetic patients; and other effects, including possible impact on sexual function. This adverse effect profile reflects propranolol's broad pharmacological action as a non-selective beta-adrenergic blocker, affecting multiple body systems simultaneously.

The author emphasizes that β2 receptor blockade can cause bronchospasm, making propranolol contraindicated in patients with asthma and other obstructive pulmonary diseases.

Propranolol no longer has valid patent protection for the original molecule. The first generic version of propranolol hydrochloride was approved in the United States in July 1985, indicating that the original patent expired around that time. The medication is listed on the World Health Organization's List of Essential Medicines. However, patents for specific controlled-release formulations and combinations with other medications may still be in effect in some countries.

Beyond its original cardiovascular applications, propranolol has been used to treat various other conditions. According to the systematic review by Steenen *et al*. (2016), non-cardiovascular indications for propranolol include migraine prophylaxis, where it provides reduction in frequency, intensity, and duration of episodes; essential tremor, offering significant symptom improvement in 40-70% of patients; performance anxiety, with reduction of tachycardia, tremors, and other physiological anxiety symptoms; social phobia and generalized anxiety disorder; and hyperthyroidism, helping control adrenergic symptoms such as tachycardia and tremor. This therapeutic versatility illustrates how propranolol's beta-adrenergic blocking mechanism influences various physiological systems, allowing its use in seemingly unrelated conditions that share pathophysiological pathways involving excessive sympathetic activation.

One of the most surprising and recent applications is in treating infantile hemangiomas. Léauté-Labrèze *et al*. (2015) accidentally discovered this indication and conducted controlled studies demonstrating propranolol's efficacy, leading to its specific approval for this condition by the FDA in 2014, establishing it as a first-line treatment for complicated hemangiomas in infants.

1.1.7. DISULFIRAM

Disulfiram was initially developed for treating chronic alcoholism. According to the historical review by Suh *et al*. (2006), this medication was the first approved specifically to combat alcohol dependence, acting as an aversive agent through inhibition of the aldehyde dehydrogenase enzyme. This inhibition prevents adequate metabolism of acetaldehyde—alcohol's toxic metabolite—causing its levels to accumulate in the body. Consequently, when patients consume alcoholic beverages, the "disulfiram effect" or "Antabuse reaction" occurs,

characterized by symptoms such as nausea, vomiting, facial flushing, and tachycardia, creating a conditioned aversion to alcohol.

Clinical studies and systematic reviews reinforce that disulfiram's efficacy is enhanced when administration is supervised. For example, the meta-analysis by Skinner *et al*. (2014) showed that open studies—where direct supervision is ensured—yielded better results in maintaining abstinence compared to blinded studies, where the medication's "threat" effect becomes diluted across groups. Additionally, Johnson (2014) clarifies the biochemical mechanisms underlying this action, detailing how blocking acetaldehyde conversion contributes to the medication's therapeutic effect.

Furthermore, the comprehensive review by Kalra *et al*. (2014) discusses various aspects of disulfiram use in the treatment of alcohol dependence, including monitoring issues, treatment adherence, and the adverse effect profile, thereby complementing the understanding of both the mechanism of action and the practical challenges in clinical application.

The drug has an interesting history of accidental discovery. According to Fuller and Gordis (2004), its effects were initially observed by chance in the late 1930s when two Danish researchers, Jens Hald and Erik Jacobsen, were investigating disulfiram's use as a treatment for parasitic infections and noticed they developed unpleasant symptoms after consuming alcohol. The substance was officially introduced as a medication for treating alcoholism in 1948 in Denmark. In the United States, the FDA approved disulfiram in 1951 under the trade name Antabuse, making it the first medication formally approved for alcohol dependence treatment.

It presents a side effect profile that extends beyond the acute reaction resulting from alcohol consumption. According to the systematic analysis by Chick (2020), adverse effects that occur independently of alcohol consumption include neurological effects, such as drowsiness, fatigue, headache, and with prolonged use, peripheral neuropathy; psychiatric effects, manifested by psychotic alterations in predisposed individuals, although this is rare; hepatic effects, characterized by elevated liver enzymes and drug-induced hepatitis; dermatological effects, presenting as skin eruptions; and endocrine effects, notably reduced libido and impotence. This spectrum of adverse effects reflects disulfiram's systemic action in the organism and its interference with multiple metabolic pathways, important factors to consider during clinical evaluation and monitoring of patients undergoing alcohol dependence treatment with this medication.

Hepatotoxicity is considered the most serious adverse effect, with an estimated incidence of approximately 1 case per 25,000 patient-years of treatment, accompanied by isolated reports of fulminant hepatic failure, which reinforces the recommendation for regular hepatic monitoring during treatment.

Recent studies using real-world data from the FAERS system corroborate the complexity of disulfiram's safety profile and emphasize the importance of rigorous clinical supervision (Luo *et al*., 2024). Moreover, rare case reports, such as methemoglobinemia associated with disulfiram use, expand the spectrum of adverse events that may occur even without alcohol consumption (Gajree & Khan, 2021).

Disulfiram no longer has valid patent protection for its original formulation, as this patent expired decades ago, allowing for its global commercialization as a generic medication. However, innovation efforts remain active, seeking to improve the drug's pharmacokinetic profile and expand therapeutic applications. Recent innovations, for example, demonstrate the development of polymeric nanoparticle-based formulations aimed at increasing stability and prolonging disulfiram's half-life, thereby enabling its utilization in emerging areas such as cancer treatment (Wang, Wang, & Bian, 2017; European Patent Office, 2024). Additionally, analyses on specialized platforms reveal an active patent portfolio encompassing advanced delivery systems, therapeutic combinations, and corroborating continued interest in disulfiram revalorization through innovative approaches (Synapse, 2025). Such initiatives demonstrate how intellectual property dynamics in the pharmaceutical industry can drive the reuse and evolution of classic medications, even after the original patent has expired.

Beyond alcoholism treatment, disulfiram has demonstrated potential for various other applications. In oncology, it exhibits antineoplastic activity through tumor growth inhibition in various cancer types, including breast, prostate, pancreatic, and glioblastoma, via multifaceted mechanisms. These mechanisms include forming copper complexes that induce the degradation of proteins essential for tumor maintenance (Skrott *et al*., 2017). Regarding antimicrobial properties, studies reveal its efficacy against resistant bacteria, including methicillin-resistant

Staphylococcus aureus (MRSA) strains (Peniche, Oliveira *et al*., 2021). The same research group identified relevant antiparasitic activity with the potential to act against protozoans such as Giardia lamblia and Trichomonas vaginalis. In the context of chemical dependencies, beyond alcoholism, disulfiram shows potential in cocaine dependence treatment through modulation of dopamine β-hydroxylase enzyme activity, altering dopaminergic response, and favoring abstinence (Gaval-Cruz, M., & Weinshenker, D., 2009). Finally, research suggests that this compound exhibits anti-HIV activity by acting on HIV latency reactivation via viral proteinase inhibition and transcription induction mechanisms, without promoting global T-lymphocyte activation (Lee *et al*., 2019; Xing *et al*., 2011). This pharmacological versatility positions disulfiram as a molecule of multidisciplinary interest in contemporary medicine.

Additionally, a recent review comprehensively compiled these emerging applications, highlighting the challenges and perspectives for repositioning disulfiram in oncology, infections, and dependencies (Lu, Yang, Zhou, & Dong, 2023).

1.1.8. VALPROIC ACID

Valproic acid was discovered by accident when used as an organic solvent in laboratory experiments, and its anticonvulsant activity was subsequently identified. Its first therapeutic indication was for epilepsy treatment, specifically for controlling absence seizures (petit mal), generalized tonic-clonic seizures (grand mal), and complex partial seizures. As highlighted by Löscher (2002), valproic acid acts by increasing the levels of gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter, in the brain, while also blocking voltage-dependent sodium channels and modulating T-type calcium currents, thereby providing a broad spectrum of antiepileptic activity.

It was first synthesized in 1882 by American chemist Beverly S. Burton, but its anticonvulsant properties were only discovered accidentally in 1963 by French researcher Pierre Eymard. According to Perucca (2002), valproic acid was first introduced to the pharmaceutical market in France in 1967 under the trade name Depakine. FDA approval in the United States occurred later, in 1978, under the trade name Depakene. In the early 1980s, sodium divalproex was developed, an enteric formulation that was more stable and caused fewer gastrointestinal effects.

A significant side effect profile requires careful monitoring. According to Genton, Semah, and Trinka (2006), the most common adverse effects include gastrointestinal effects (nausea, vomiting, dyspepsia, diarrhea), weight gain, transient hair loss, tremors, sedation, and drowsiness. Similarly, studies have demonstrated that hepatotoxicity is a serious adverse effect—with a risk of fatal hepatic failure, especially in children under 2 years—emphasizing the importance of periodic laboratory monitoring (Xu *et al*., 2019). Other severe effects reported include pancreatitis, thrombocytopenia, hyperammonemic encephalopathy, and polycystic ovary syndrome. Furthermore, valproic acid teratogenicity is one of the most concerning issues, as it is associated with a significantly increased risk of congenital malformations, including neural tube defects, a fact reinforced by studies investigating the drug's effects during pregnancy (Mawhinney *et al*., 2012).

Valproic acid no longer has valid patent protection for the basic molecule and is available as a generic medication globally. According to FDA Orange Book data, the patents for Depakote ER (divalproex sodium, NDA 021168) expired on December 18, 2018 (US 6528090, US 6511678) and June 18, 2019 (US 6713086, US 6720004) (U.S. Food and Drug Administration, 2025). Currently, various immediate-release and extended-release generic formulations are available on the market, significantly reducing treatment costs and expanding access to medication (DrugPatentWatch, 2025).

Beyond its original use as an anticonvulsant, valproic acid has demonstrated efficacy in various other clinical contexts. According to the review by Cipriani *et al*. (2013), its non-epileptic applications include treatment of acute manic episodes and prevention of relapses in bipolar patients, prophylactic management of migraine and cluster headaches, interventions for personality disorders, especially borderline, and reduction of agitation and aggressiveness in patients with dementia.

Recent research has expanded the scope of its applications, particularly through its action as a histone deacetylase (HDAC) inhibitor. Studies indicate this property may have anticancer effects, and it is being investigated in tumors such as gliomas (Han *et al*., 2021). Additionally, there is

growing interest in its epigenetic action in modulating neurodegenerative diseases, including Parkinson's and Huntington's (Ximenes *et al*., 2012).

Another field of study involves valproic acid's potential in managing autism spectrum disorders. Animal models have explored mechanisms by which it influences neurological development and control of repetitive behaviors and emotional outbursts (Devahuti *et al*., 2020). However, studies suggest that prenatal exposure may be associated with increased autism risk, highlighting the need for additional research to understand its clinical effects and safety (Sivasangari *et al*., 2022).

1.1.9. THALIDOMIDE

Thalidomide was originally developed and marketed as a sedative-hypnotic and antiemetic for treating insomnia, anxiety, and morning sickness in pregnant women. According to Vargesson (2015), thalidomide was initially promoted as a "completely safe" medication for everyone, including pregnant women, due to its apparent absence of acute toxicity and the impossibility of causing overdose—characteristics that distinguished it from barbiturates available at the time. The medication quickly became popular, particularly among pregnant women, due to its efficacy in controlling nausea and vomiting associated with the first trimester of pregnancy.

Thalidomide was first synthesized in 1954 by the German pharmaceutical company Chemie Grünenthal. According to the historical study by Lenz (1988), the medication was introduced to the market in West Germany in October 1957 under the trade name Contergan and subsequently marketed in more than 40 countries worldwide, including the United Kingdom, Australia, and Canada, under various trade names. Notably, the medication never received approval in the United States, thanks to the persistence of FDA reviewer Frances Kelsey, who questioned the drug's safety, especially the absence of studies on its placental passage. In 1961, after association with severe congenital malformations, thalidomide was withdrawn from the global market, representing one of the greatest pharmaceutical disasters in modern history.

The most devastating and notorious side effect of thalidomide is its teratogenicity, which resulted in one of the greatest pharmacological tragedies in history. As extensively documented, including by Matthews & McCoy (2003) and in more recent reviews such as Vargesson (2015), exposure to the medication during pregnancy, particularly between 20 and 36 days after conception, resulted in the birth of more than 10,000 children with severe congenital malformations, collectively known as "thalidomide syndrome" or "thalidomide embryopathy." The most common characteristics of this syndrome include phocomelia (shortening or absence of limbs), amelia (complete absence of limbs), ear and eye malformations, cardiac defects, and malformations of the gastrointestinal and urogenital tracts. Beyond its teratogenicity, thalidomide presents other important adverse effects, including peripheral neuropathy (which may be irreversible in 25-30% of patients), drowsiness, constipation, skin eruptions, neutropenia, and increased risk of venous thromboembolism, especially when combined with dexamethasone (Matthews & McCoy, 2003).

The patent situation for thalidomide is complex and unique (Thalidomide—A Revival Story, 1999). According to Okafor (2003) and Haslett *et al*. (2005), after its reintroduction for new indications, Celgene Corporation obtained patents for the medication in the United States in the mid-1990s, not for the molecule itself (which was in the public domain), but for methods of use and controlled distribution systems (U.S. Patent No. 5,715,309, 1998; U.S. Patent No. 6,248,362, 2001). The principal patent for use in treating erythema nodosum leprosum (under the trade name Thalomid) expired in 2014 (U.S. Patent No. 5,715,309, 1998), and for multiple myeloma, in 2019 (U.S. Patent No. 6,248,362, 2001; Palumbo *et al*., 2006). Currently, generic versions are available in some markets but under strictly controlled distribution systems due to teratogenic risk (Pharsight GreyB, 2023). Thalidomide and its analogs (lenalidomide, pomalidomide) remain protected by method-of-use and distribution patents in some countries (Sarpatwari *et al*., 2018).

Despite its tragic history, thalidomide found its way back into modern medicine for several indications. According to the comprehensive review by Franks *et al*. (2004), current therapeutic applications include erythema nodosum leprosum (ENL), which was the first indication approved after reintroduction, leveraging its anti-inflammatory and immunomodulatory properties; multiple myeloma, where it is used in combination with dexamethasone for treating newly diagnosed or refractory patients, as demonstrated by Singhal *et al*. (1999); graft-versus-host disease (GVHD), a

condition that may occur after bone marrow transplantation, as evidenced by Browne *et al*. (2000); aphthous ulcers and Behçet's disease, applications documented by Hamuryudan *et al*. (1998); and discoid and cutaneous lupus erythematosus, according to more recent studies conducted by Verdelli *et al*. (2022). This therapeutic rehabilitation represents a remarkable case in pharmacology, where a medication initially withdrawn from the market for its devastating teratogenic effects was rediscovered and repositioned with rigorous safety protocols to treat serious conditions with limited therapeutic options, demonstrating how understanding molecular mechanisms can transform a harmful agent into a valuable therapeutic tool.

More recent investigations explore its potential in autoimmune diseases, various types of hematological and solid cancers, myelodysplastic syndromes, and HIV-associated complications (Kaplan *et al*., 2000), such as wasting syndrome and oral ulcers. Its mechanism of action involves immunomodulatory, anti-inflammatory, and anti-angiogenic properties.

1.1.10. DEXAMETHASONE

Dexamethasone was developed as a long-acting synthetic corticosteroid with potent anti-inflammatory and immunosuppressive properties (Schäcke *et al*., 2002). As described by Czock *et al*. (2005), dexamethasone's original applications included treating acute and chronic inflammatory conditions such as rheumatoid arthritis, bronchial asthma, severe allergic reactions, and autoimmune disorders. The medication acts by binding to glucocorticoid receptors in the cellular cytoplasm and, after translocation to the nucleus, regulates transcription of various genes, resulting in decreased production of pro-inflammatory cytokines and suppression of inflammatory cell migration to affected tissues (Barnes, 2006; Coutinho & Chapman, 2011). Dexamethasone is approximately 25 times more potent than cortisone in its anti-inflammatory activities (Liu *et al*., 2013).

Dexamethasone was first synthesized in 1957 by researchers at Merck & Co. According to Benedek (2011), the medication received FDA approval in the United States in 1958. It rapidly became one of the most widely used corticosteroids in clinical practice due to its high potency, long duration of action, and reduced sodium retention compared to other corticosteroids available at the time. By the 1960s, dexamethasone was widely available in the global market, used in various formulations, including oral, intravenous, intramuscular, topical, and ophthalmic, which significantly expanded its use across different medical specialties.

Dexamethasone, like other corticosteroids, presents numerous side effects, especially with prolonged use. According to the systematic review by Liu *et al*. (2013), the most common adverse effects include metabolic effects such as hyperglycemia, steroid-induced diabetes, weight gain, and cushingoid fat distribution; musculoskeletal effects manifested by corticosteroid-induced osteoporosis muscle weakness, and myopathy; psychiatric effects encompassing mood alterations, insomnia, psychosis, and delirium; suppression of the hypothalamic-pituitary-adrenal (HPA) axis, a condition that may persist for months after medication discontinuation; increased susceptibility to infections, including reactivation of latent tuberculosis and opportunistic infections; gastrointestinal effects, notably peptic ulcer, especially when used concomitantly with NSAIDs; and ophthalmological effects, particularly posterior subcapsular cataract and glaucoma. This extensive adverse effect profile reflects the potent systemic action of corticosteroids and explains why their clinical use frequently involves careful risk-benefit evaluation, with a preference for treatment regimens that use the lowest effective dose for the shortest possible time, along with regular clinical monitoring during prolonged therapy.

The severity and incidence of these effects are dose-dependent and increase significantly with treatment duration.

Dexamethasone no longer has valid patent protection for the basic molecule (Arth *et al*., 1958). According to Dave *et al*. (2017), the original patents for dexamethasone expired decades ago, and the medication is now available as a generic in virtually all global markets. However, patents exist for specific formulations and delivery systems, such as intraocular dexamethasone implants (Ozurdex) for treating diabetic macular edema and extended-release formulations for inhalation use. Patents in various countries may protect these specific formulations. However, the basic dexamethasone molecule is considered an essential medicine by the World Health Organization and is available at low cost in generic formulations.

Beyond its original anti-inflammatory and immunosuppressive applications, dexamethasone

has been used in various other clinical contexts. According to the comprehensive study by Cain & Cidlowski (2017), non-conventional applications of dexamethasone include its use in oncology, where it functions as an essential component of chemotherapeutic regimens for leukemias, lymphomas, and multiple myeloma, acting both as a direct cytotoxic agent and as an adjuvant to reduce side effects of other chemotherapeutics (Patel & Dickenson, 2016); in neurology, where Roberts *et al*. (2017) described its value in treating cerebral edema associated with brain tumors, traumatic brain injury, and stroke; in endocrinology, serving as a diagnostic tool through the dexamethasone suppression test for identifying Cushing's syndrome; in obstetrics, where it plays a crucial role in accelerating fetal lung maturation in pregnant women at risk of premature delivery, potentially saving lives of premature newborns; in anesthesiology, contributing significantly to preventing postoperative nausea and vomiting (Oliveira *et al*., 2013); and in ophthalmology, where it has demonstrated efficacy in treating conditions such as diabetic macular edema and non-infectious uveitis (Galor *et al*., 2008). This diversity of clinical applications illustrates dexamethasone's remarkable therapeutic versatility—a medication that, despite its known side effects, continues to expand its role across multiple medical specialties due to its potent mechanism of action and the deepening scientific understanding of how it modulates inflammatory and immune responses.

In 2020, dexamethasone gained worldwide attention when the RECOVERY study demonstrated significant mortality reduction in patients with severe COVID-19 requiring oxygen therapy or mechanical ventilation, establishing it as the first proven effective treatment for severe cases of the disease.

1.1.11. HYDROXYCHLOROQUINE

Hydroxychloroquine was developed as a chloroquine derivative, with the primary objective of being a safer and more effective antimalarial agent. According to Ben-Zvi *et al*. (2012), the original application of hydroxychloroquine was the treatment and prevention of malaria, particularly caused by chloroquine-sensitive Plasmodium strains. Its antimalarial mechanism of action involves concentration in the parasite's acidic digestive vacuole, where it interferes with hemoglobin degradation and heme group detoxification, resulting in parasite death. Hydroxychloroquine presents advantages over chloroquine, including lower ocular toxicity and a generally better safety profile while maintaining comparable efficacy against malaria.

Hydroxychloroquine was first synthesized in 1946 at Sanofi Research laboratories. According to historical research by Rainsford *et al*. (2015), the medication was approved by the FDA in 1955, initially as an antimalarial agent, under the trade name Plaquenil. It was introduced to the market as a less toxic alternative to chloroquine, particularly with a lower risk of retinopathy and other adverse effects. During the 1950s and 1960s, while it was widely used for malaria prevention and treatment, observations began to emerge about its beneficial effects on patients with rheumatic diseases, which would subsequently lead to its approval for these new indications.

Although generally considered safer than chloroquine, hydroxychloroquine still presents a significant side effect profile. According to the systematic review by Ruiz-Irastorza *et al*. (2010), the most common adverse effects include gastrointestinal effects, characterized by nausea, vomiting, abdominal pain, and diarrhea, which frequently represent the first signs of medication intolerance; dermatological effects, manifested as skin eruptions, pruritus, and hyperpigmentation, the latter being particularly notable for its potential persistence even after therapy discontinuation; and neurological effects, expressed primarily as headache, dizziness, and insomnia, which can significantly affect patients' quality of life. These adverse effects, although generally less severe than those of chloroquine, still merit careful clinical attention, especially in long-term treatments as occurs in autoimmune diseases, where the balance between therapeutic efficacy and medication safety becomes particularly critical for treatment adherence and therapeutic intervention success.

The most serious and feared adverse effect is hydroxychloroquine retinopathy, which can lead to irreversible vision loss. According to Marmor *et al*. (2016), the risk is dose-dependent, being higher in patients who take doses exceeding 5 mg/kg/day or have cumulative use for more than 5 years. Another important adverse effect is cardiotoxicity, which manifests as conduction disturbances, cardiomyopathy, and QT interval prolongation, with potential risk of fatal arrhythmias, particularly in patients with pre-existing cardiac risk factors or when used in combination with other medications that prolong the QT interval.

Hydroxychloroquine no longer has valid

patent protection for the original molecule. Hydroxychloroquine patents expired decades ago, and the medication is widely available as a generic worldwide. Sanofi, the original manufacturer of Plaquenil, continues to produce the brand version; however, it holds only a minority share in the global market, which is dominated by generic formulations. The medication is included in the World Health Organization's List of Essential Medicines due to its fundamental role in treating certain rheumatic diseases and is still considered important in some antimalarial treatment protocols, especially in regions where chloroquine resistance is not prevalent (Shippey, Wagler, & Collamer, 2018; D'Acquarica & Agranat, 2020; WHO, 2011).

Beyond its original use as an antimalarial, hydroxychloroquine has established itself as a fundamental treatment for various autoimmune diseases. According to the comprehensive review by Schrezenmeier & Dörner (2020), non-malarial applications of hydroxychloroquine include systemic lupus erythematosus (SLE), where it is considered baseline therapy for virtually all patients, reducing disease activity, organ damage, and mortality, as demonstrated by Fanouriakis *et al*. (2019); rheumatoid arthritis, where it is used as monotherapy in mild cases or in combination with other disease-modifying antirheumatic drugs, following guidelines established by Smolen *et al*. (2019); Sjögren's syndrome, providing relief from musculoskeletal manifestations and fatigue, benefits documented by Vivino *et al*. (2016); dermatomyositis and polymyositis, where it acts by modulating the dysregulated immune response; cutaneous porphyria tarda, as evidenced in studies by Singal (2019), where it assists in porphyrin metabolism; and juvenile idiopathic arthritis, expanding the spectrum of its use to the pediatric population. This therapeutic versatility reflects how a medication initially developed for antiparasitic purposes found significant applications in immune-mediated conditions, thanks to its mechanisms of action that modulate conserved inflammatory pathways involved in various autoimmune pathological processes.

In 2020, hydroxychloroquine received worldwide attention when it was investigated as a potential treatment for COVID-19 (Meo *et al*., 2020), although subsequent studies did not confirm its efficacy for this indication. More recent research has explored its possible metabolic effects (improvement of insulin sensitivity and lipid profile) (Rempenault *et al*., 2018), antiplatelet, and antineoplastic properties, suggesting potential future applications in diabetes, metabolic syndrome, and some types of cancer.

2. METHODS

This study presents an exploratory bibliometric analysis of scientific research patterns related to the repurposing of drugs for cancer treatment. The research was conducted from January 1 to May 10, 2025, encompassing all available periods across the databases. A quantitative search was performed across multiple databases to map and compare the volume of scientific literature on eleven off-patent medications with anticancer potential, aiming to identify patterns in the distribution of scientific attention and potential knowledge gaps in this research field.

2.1. Methods

This bibliometric analysis was conducted across five databases: Google Scholar (https://scholar.google.com/), BVS (Virtual Health Library, https://bvsalud.org/), PubMed (https://pubmed.ncbi.nlm.nih.gov/), NIH (National Institutes of Health, https://www.nih.gov/), and Science.gov (https://www.science.gov/). These databases were selected for their comprehensiveness, relevance to health research, open access availability, and coverage of both academic literature and governmental/institutional publications related to cancer.

Search terms focused on investigating the therapeutic potential of existing medications (drug repurposing) for cancer treatment. The following term combinations were used consistently across all databases:

Ivermectin AND cancer

Fenbendazole AND cancer

Mebendazole AND cancer

Albendazole AND cancer

Metformin AND cancer

Propranolol AND cancer

Disulfiram AND cancer

Valproate AND cancer

Thalidomide AND cancer

Dexamethasone AND cancer

Hydroxychloroquine (with Zinc) AND cancer

Disulfiram (with Copper) AND cancer

Ivermectin AND cancer treatment

Fenbendazole AND cancer treatment

Mebendazole AND cancer treatment

Albendazole AND cancer treatment

Metformin AND cancer treatment

Propranolol AND cancer treatment

Disulfiram AND cancer treatment

Valproate AND cancer treatment

Thalidomide AND cancer treatment

Dexamethasone AND cancer treatment

Hydroxychloroquine (with Zinc) AND cancer treatment

Disulfiram (with Copper) AND cancer treatment

The Boolean operator "AND" was used to connect specific medications with cancer-related terms. This approach enabled the identification of studies investigating these approved medications for alternative oncological applications, a field known as drug repurposing. The search strategy was applied consistently across databases, with minimal adaptations to accommodate platform-specific syntactic requirements.

2.2.1. Data Collection and Recording Procedures

For this bibliometric study, all search results obtained from the databases were accepted. No specific filters or exclusion criteria were applied, as the primary objective was to quantify and analyze the volume of scientific production related to each investigated medication within the context of cancer treatment.

This methodological approach was chosen to provide a comprehensive overview of the current scientific landscape regarding the repurposing of these specific medications for oncological use without restricting the analytical scope. The methodology enabled the identification of literature trends, medications with higher or lower associated research volumes, and the temporal evolution of publications in this field.

For each search term combination, only the total number of results from each database was recorded. This straightforward quantitative approach was chosen to facilitate a direct comparison of the available scientific literature volumes for each medication investigated in cancer treatment.

2.2.2. Statistical Analyses Performed

Two scripts (CODE 1 and CODE 2, provided in the appendix) were developed with the assistance of artificial intelligence to analyze the values presented in Table 1. CODE 1 presents results in textual format, while CODE 2 generates graphical outputs.

2.2.2.1. Basic Descriptive Analysis

- Measures of central tendency and dispersion

2.2.2.2. Ranking and Volume Analysis

- Hierarchical ordering
- Aggregation by pharmacological classes

2.2.2.3. Proportion and Specificity Analysis

- Ratio calculations
- Categorical comparisons

2.2.2.4. Correlation Analysis

- Pearson coefficient
- Correlation strength classification

2.2.2.5. Contribution and Concentration Analysis

- Percentage distribution
- Herfindahl Index

2.2.2.6. Data Source Efficiency Analysis

- Proportional distribution

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1. Basic Descriptive Analysis

Total number of entries: 24

Total sum of results: 3,226,066

Average results per entry: 134,419.42

Median of results: 42,362.5

Standard deviation: 266,145.27

Minimum value: 18,607

Maximum value: 1,295,666

The high standard deviation (266,145.27) compared to the mean (134,419.42) and the substantial difference between minimum (18,607) and maximum (1,295,666) values indicate an extremely asymmetric distribution. This asymmetry suggests that some medications receive considerably more scientific attention than others, creating a highly unbalanced research landscape. The median (42,362.5) being significantly lower than the mean confirms this unequal distribution, with few medications dominating publication volumes.

3.1.2 Ranking of Drugs by Total Publication Volume

Publication distribution shows extreme concentration among a few medications. Dexamethasone (1,538,058 publications) and metformin (697,172 publications) together represent nearly 70% of total publications, while the six least-researched drugs combined account for approximately 10%. This disparity indicates unequal allocation of research resources and attention, possibly reflecting the greater clinical history of these dominant medications or their prior establishment as adjuvant therapies in oncology. Figure 1 illustrates this analysis.

Ranking by total publication volume (general + treatment):

1. **Dexamethasone**: 1,538,058 publications (1,295,666 general, 242,392 treatment)
2. **Metformin**: 697,172 publications (544,873 general, 152,299 treatment)
3. **Thalidomide**: 218,059 publications (148,413 general, 69,646 treatment)
4. **Valproate**: 185,787 publications (122,942 general, 62,845 treatment)
5. **Propranolol**: 157,371 publications (115,566 general, 41,805 treatment)
6. **Disulfiram**: 88,496 publications (56,162 general, 32,334 treatment)
7. **Ivermectin**: 80,772 publications (53,131 general, 27,641 treatment)
8. **Albendazole**: 67,401 publications (42,920 general, 24,481 treatment)
9. **Mebendazole**: 52,324 publications (31,124 general, 21,200 treatment)
10. **Disulfiram** (with Copper): 50,463 publications (27,052 general, 23,411 treatment)
11. **Hydroxychloroquine** (with Zinc):50,368 publications (28,747 general, 21,621 treatment)
12. **Fenbendazole**: 39,795 publications (21,188 general, 18,607 treatment)

3.1.3. Analysis by Pharmacological Classes

Pharmacological classes show marked differences in both volume and research focus. Corticosteroids (18.71%) have a high representation in absolute volume but a low proportion of treatment-specific research, while antidiabetics (27.95%) show a moderate proportion. In contrast, antimalarials (75.21%), anti-alcoholics (66.99%), and antiparasitics (61.96%) present much higher proportions of studies specifically focused on treatment. This suggests that these latter classes, although less studied in absolute terms, are being investigated more directly for specific oncological applications, possibly indicating emerging interest in these classes as anticancer therapies. Figure 2 illustrates this analysis.

- **Group: Corticosteroids**
  Total publications: 1,538,058
  General publications: 1,295,666
  Treatment publications: 242,392
  Treatment/general ratio: 18.71%
  Drugs in the group: Dexamethasone

- **Group: Antidiabetics**
  Total publications: 697,172
  General publications: 544,873
  Treatment publications: 152,299
  Treatment/general ratio: 27.95%
  Drugs in the group: Metformin

- **Group: Antiparasitics**
  Total publications: 240,292
  General publications: 148,363
  Treatment publications: 91,929
  Treatment/general ratio: 61.96%
  Drugs in the group: Ivermectin, Fenbendazole, Mebendazole, Albendazole

- **Group: Immunosuppressants**
  Total publications: 218,059
  General publications: 148,413
  Treatment publications: 69,646
  Treatment/general ratio: 46.93%
  Drugs in the group: Thalidomide

- **Group: Anticonvulsants**
  Total publications: 185,787
  General publications: 122,942

Treatment publications: 62,845
Treatment/general ratio: 51.12%
Drugs in the group: Valproate

- **Group: Beta-blockers**
  Total publications: 157,371
  General publications: 115,566
  Treatment publications: 41,805
  Treatment/general ratio: 36.17%
  Drugs in the group: Propranolol

- **Group: Antialcoholics**
  Total publications: 138,959
  General publications: 83,214
  Treatment publications: 55,745
  Treatment/general ratio: 66.99%
  Drugs in the group: Disulfiram, Disulfiram (with Copper)

- **Group: Antimalarials**
  Total publications: 50,368
  General publications: 28,747
  Treatment publications: 21,621
  Treatment/general ratio: 75.21%
  Drugs in the group: Hydroxychloroquine (with Zinc)

3.1.4. Specificity Analysis (Treatment/General)

A clear inverse relationship exists between total publication volume and the proportion of treatment-specific research. Less-studied medications such as fenbendazole (87.82%), disulfiram with copper (86.54%), and hydroxychloroquine with zinc (75.21%) show the highest proportions of treatment-focused studies. In contrast, dexamethasone, despite having the largest total volume, exhibits the lowest proportion (18.71%). This relationship indicates that although less popular medications receive reduced general attention, existing research is potentially more targeted toward their therapeutic potential in cancer, possibly reflecting more recent and focused interest in their anticancer properties. Figure 3 illustrates this analysis.

Proportion of 'treatment' results relative to 'general' results:

Fenbendazole: 87.82%
(18,607 treatment / 21,188 general)

Disulfiram (with Copper): 86.54%
(23,411 treatment / 27,052 general)

Hydroxychloroquine (with Zinc): 75.21%
(21,621 treatment / 28,747 general)

Mebendazole: 68.11%
(21,200 treatment / 31,124 general)

Disulfiram: 57.57%
(32,334 treatment / 56,162 general)

Albendazole: 57.04%
(24,481 treatment / 42,920 general)

Ivermectin: 52.02%
(27,641 treatment / 53,131 general)

Valproate: 51.12%
(62,845 treatment / 122,942 general)

Thalidomide: 46.93%
(69,646 treatment / 148,413 general)

Propranolol: 36.17%
(41,805 treatment / 115,566 general)

Metformin: 27.95%
(152,299 treatment / 544,873 general)

Dexamethasone: 18.71%
(242,392 treatment / 1,295,666 general)

3.1.5. Correlation Analysis Between Databases

Correlation patterns between databases reveal important differences in indexing criteria and coverage. BVS exhibits a strong correlation with several databases, particularly with Science.gov ($r = 0.9205$), indicating similar indexing patterns. In contrast, Google Scholar and PubMed present surprisingly low correlations (0.1562), indicating significant differences in their indexing approaches. This pattern has important implications for bibliographic research strategies: researchers should consult multiple databases to ensure comprehensive coverage, as different databases capture different subsets of the literature. Figure 4 illustrates this analysis.

Correlations between databases (Pearson coefficient):

BVS vs Science.gov: 0.9205 (Very strong)

Google Scholar vs BVS: 0.8492 (Very strong)

BVS vs NIH: 0.8021 (Very strong)

Google Scholar vs NIH: 0.7916 (Strong)

PubMed vs Science.gov: 0.7558 (Strong)

Google Scholar vs Science.gov: 0.7116 (Strong)

NIH vs Science.gov: 0.6454 (Strong)

BVS vs PubMed: 0.5871 (Moderate)

PubMed vs NIH: 0.3231 (Weak)

Google Scholar vs PubMed: 0.1562 (Very weak)

3.1.6. Relative Contribution Analysis

Percentage contribution of each drug to the total publications:

Dexamethasone: 47.68%

Metformin: 21.61%

Thalidomide: 6.76%

Valproate: 5.76%

Propranolol: 4.88%

Disulfiram: 2.74%

Ivermectin: 2.50%

Albendazole: 2.09%

Mebendazole: 1.62%

Disulfiram (with Copper): 1.56%

Hydroxychloroquine (with Zinc): 1.56%

Fenbendazole: 1.23%

Herfindahl Index (research concentration): 0.2870

Interpretation: Highly concentrated

The Herfindahl Index of 0.2870 confirms a high level of research concentration, classified as "highly concentrated." Two medications—dexamethasone (47.68%) and metformin (21.61%)—dominate the landscape, representing nearly 70% of all publications. This concentration suggests a notable imbalance in research focus, which may limit the exploration of the therapeutic potential of alternative medications. The high degree of concentration raises questions about factors driving research priorities and suggests opportunities for diversification in future studies.

3.1.7. Database Search Efficiency

Google Scholar (64.39%) and Science.gov (33.63%) collectively account for 98% of all results, while the other three databases contribute approximately 2%. This disparity suggests that Google Scholar and Science.gov were the most comprehensive sources for drug repurposing research in oncology within this study. For researchers with limited resources, focusing on these two databases would provide the most efficient coverage. However, less representative databases may contain unique publications not found in the primary sources, highlighting the value of a comprehensive approach when feasible. Figure 5 illustrates this analysis.

Contribution of each database to the total results:

Google Scholar: 2,077,425 results (64.39% of total)

Science.gov: 1,085,038 results (33.63% of total)

BVS: 50,821 results (1.58% of total)

PubMed: 50,591 results (1.57% of total)

NIH: 1,316 results (0.04% of total)

3.2. Discussions

3.2.1 Distribution Patterns in Repurposed Drug Research

The striking asymmetry in research distribution revealed by the analysis (SD = 266,145.27 compared to the mean = 134,419.42) reflects a fundamental imbalance in scientific attention toward repurposed medications for cancer treatment. This pattern aligns with what Pantziarka *et al*. (2021) termed the "popularity bias" in drug repurposing research, where established medications with known anticancer properties receive disproportionate attention compared to emerging candidates.

The dominance of dexamethasone and metformin, collectively representing nearly 70% of all publications, demonstrates the influence of clinical integration on research interest. Dexamethasone has been a standard component of many chemotherapy regimens since the 1980s (Weissman *et al*., 2019), while metformin's potential anticancer properties have been extensively studied following Bodmer *et al*.'s (2010) landmark epidemiological study, which showed a reduced incidence of cancer in diabetic patients taking metformin. This finding supports Verbaanderd *et al*.'s (2017) observation that research momentum in drug repurposing is often driven by early clinical observations rather than mechanistic rationales.

3.2.2 Pharmacological Class Variation and Research Focus

The significant differences in both volume and research focus across pharmacological classes reflect varying stages of evidence development. The high representation of corticosteroids (18.71%) and antidiabetics (27.95%), alongside their low proportion of treatment-specific research, suggests these classes have reached a mature research stage where their mechanisms and applications are

broadly studied beyond specific cancer treatment protocols.

Conversely, antimalarials (75.21%), antialcoholics (66.99%), and antiparasitics (61.96%) exhibit a significantly higher proportion of treatment-focused research, despite having lower absolute publication volumes. This pattern aligns with Pushpakom *et al.*'s (2019) description of the drug repurposing research lifecycle, where emerging candidates initially generate targeted mechanism-of-action studies before being integrated more broadly into cancer research. These classes appear to be in earlier stages of the repurposing research cycle, with investigations more specifically directed toward anticancer applications.

3.2.3 The Inverse Relationship Between Volume and Specificity

The identified inverse relationship between total publication volume and treatment specificity ratio represents a novel observation in bibliometric studies of drug repurposing. Medications with lower overall research attention, such as fenbendazole (87.82%) and disulfiram with copper (86.54%), demonstrate remarkably higher proportions of cancer treatment-specific research compared to more established drugs like dexamethasone (18.71%).

This finding suggests a systematic pattern in how repurposed drugs evolve in the research landscape. Newer candidates in the repurposing pipeline appear to generate more focused, hypothesis-driven research specifically targeting cancer applications, while established medications become incorporated into broader research agendas. This pattern aligns with Cha et al.'s (2018) framework, which describes how repurposed drugs transition from the "candidate exploration" to the "clinical integration" phases, with research becoming progressively more diversified as a drug advances through this continuum.

3.2.4 Database Indexing Patterns and Research Strategy Implications

The correlation analysis between databases reveals important considerations for systematic review methodologies in repurposed drug research. The strong correlation between BVS and Science.gov (r = 0.9205) suggests a significant overlap in indexing patterns, while the surprisingly weak correlation between Google Scholar and PubMed (r = 0.1562) indicates substantial differences in coverage.

These findings have direct implications for systematic review protocols in drug repurposing research. The results challenge the assumption that PubMed alone provides comprehensive coverage, supporting Martín-Martín *et al.*'s (2021) recommendation for multi-database approaches in comprehensive literature reviews. For researchers conducting systematic reviews on repurposed drugs, the findings suggest that combining Google Scholar and Science.gov would provide the most efficient coverage (98% of all results), with specialized databases adding marginal but potentially unique content.

3.2.5 Research Concentration and Funding Implications

The high Herfindahl Index (0.2870) indicates substantial concentration in the research landscape, raising important questions about research funding allocation and the allocation of scientific attention. This concentration pattern aligns with Showalter *et al.*'s (2020) critique of research funding distribution in drug repurposing, which tends to follow established pathways rather than encourage the exploration of novel candidates.

The dominance of dexamethasone (47.68%) and metformin (21.61%) suggests that factors beyond anticancer efficacy alone may disproportionately influence research interest. Institutional factors, funding priorities, and clinical familiarity likely play significant roles in directing research attention. This observation supports Bloom *et al.*'s (2020) argument that research in drug repurposing is often subject to path dependency, where early success creates self-reinforcing cycles of attention and funding.

3.2.6 Potential of Neglected Candidates

The highly skewed distribution identified in the analysis suggests significant untapped potential in less-studied candidates. Medications like fenbendazole, which demonstrated the highest treatment specificity ratio (87.82%), may represent underexplored opportunities despite promising mechanistic evidence. This aligns with Bertolini *et al.*'s (2015) observation that many promising repurposed drug candidates remain "orphaned" in the research pipeline due to insufficient attention, rather than a lack of efficacy.

The antiparasitic class as a whole

demonstrates a high treatment specificity ratio (61.96%), indicating a focused research interest in their anticancer mechanisms, despite relatively modest publication volumes. Recent mechanistic studies have identified multiple potential anticancer pathways for these medications, including microtubule disruption, modulation of autophagy, and selective toxicity in cancer cells (Tang *et al*., 2021; Nguyen *et al*., 2024). The imbalance between promising mechanistic findings and overall research volume highlights a potential opportunity for expanded investigation.

3.2.7 Research Implications Without Google Scholar

The potential exclusion of Google Scholar from bibliometric analyses in drug repurposing research would have profound methodological and interpretative implications, fundamentally altering both the scope and conclusions of such studies.

3.2.7.1 Substantial Data Loss and Coverage Reduction

Removing Google Scholar would eliminate 64.39% of all search results (2,077,425 publications), representing the most significant single source of bibliographic data in this analysis. This massive reduction would shift the research landscape from a multi-source perspective to one heavily dominated by Science.gov, which would increase from 33.63% to approximately 91.36% of the remaining dataset. Such concentration in a single database would raise concerns about the diversity and comprehensiveness of sources in bibliometric assessments.

3.2.7.2 Altered Research Distribution Patterns

The exclusion would disproportionately affect certain medications that show high representation in Google Scholar. Dexamethasone, for instance, would lose 1,110,000 of its 1,295,666 total publications (85.68%) while maintaining relatively smaller losses in specialized databases. This differential impact would substantially alter the hierarchical ranking of medications and potentially modify the concentration patterns measured by the Herfindahl Index, possibly reducing the apparent research concentration observed in the current analysis.

3.2.7.3.Enhanced Specialized Database Focus

Conversely, removing Google Scholar might strengthen the focus on more specialized, peer-reviewed literature indexed in PubMed, NIH, and BVS databases. The weak correlation between Google Scholar and PubMed ($r = 0.1562$) suggests that these sources capture different types of publications, with PubMed likely representing more rigorous, peer-reviewed research. This shift could provide a more selective view of high-quality research, potentially offering different insights into treatment specificity ratios and pharmacological class patterns.

3.2.7.4. Impact on Treatment Specificity Analysis

The inverse relationship between publication volume and treatment specificity might be affected differently across medications. Drugs with high Google Scholar representation but lower specialized database presence might show altered specificity ratios, potentially strengthening the observed pattern or revealing different relationships when focusing solely on specialized literature.

3.2.7.5 Methodological Trade-offs

Excluding Google Scholar would represent a trade-off between comprehensiveness and selectivity. While losing breadth of coverage, the analysis might gain in terms of literature quality and relevance, focusing on publications that are more likely to meet traditional academic standards. However, this approach may overlook emerging research, preprints, conference presentations, and grey literature that could be relevant for identifying early research trends in drug repurposing.

3.2.7.6 Database Correlation Implications

The removal would strengthen the relative importance of correlations between the remaining databases, particularly the strong correlation between BVS-Science.gov (0.9205), potentially indicating more consistent indexing patterns in specialized health databases compared to the broad academic coverage provided by Google Scholar.

3.2.7.7 Research Strategy Recommendations

These considerations suggest that future bibliometric studies in drug repurposing should explicitly address the inclusion criteria for databases, weighing comprehensiveness against selectivity based on research objectives. For studies focusing on established peer-reviewed evidence, excluding Google Scholar may provide more targeted insights, while comprehensive landscape mapping would benefit from its inclusion, despite potential variations in quality.

3.2.7.8. Basic Statistical Data Adjusted Without Google Scholar

Recalculating fundamental statistical parameters after excluding Google Scholar reveals significant shifts in research distribution patterns and concentration metrics.

Adjusted Basic Descriptive Statistics (Without Google Scholar):

- Total entries: 24
- Total sum of results: 1,148,641 (64.39% reduction)
- Mean results per entry: 47,860.04 (64.4% reduction)
- Median: 25,647.5 (39.5% reduction)
- Standard deviation: 48,628.91 (81.7% reduction)
- Minimum value: 17,728 (4.7% reduction)
- Maximum value: 187,658 (85.5% reduction)

Key Distribution Changes:

Variance Reduction: The standard deviation decreased from 266,145.27 to 48,628.91, indicating that Google Scholar was the primary driver of extreme asymmetry in the original dataset. The coefficient of variation decreased from 1.98 to 1.02, suggesting a more balanced distribution across medications when focusing on specialized databases.

Range Compression: The total range compressed from 1,277,059 to 169,930, primarily due to dexamethasone's reduction from 1,295,666 to 187,658 publications. Despite this compression, right-skewness persists, with the median (25,647.5) remaining below the mean (47,860.04).

Differential Medication Impact:

Highest impact: Dexamethasone (85.5% reduction), Metformin (78.7% reduction)

- **Moderate impact:** Propranolol (69.3% reduction), Disulfiram (51.7% reduction)
- **Lowest impact:** Fenbendazole (4.7% reduction), Mebendazole (37.2% reduction)

Implications: The substantial variance reduction suggests that the extreme asymmetry observed in the original analysis was significantly influenced by Google Scholar's broader indexing criteria. The adjusted dataset exhibits more moderate distribution patterns, which may better reflect peer-reviewed research in specialized databases, albeit with reduced comprehensive coverage.

Relative medication rankings remain largely consistent, indicating that Google Scholar amplifies rather than distorts underlying research distribution patterns captured by specialized databases.

Based on Table 1, calculating only the values from BVS + PubMed + NIH + Science.gov (excluding Google Scholar), the adjusted ranking would be:

1. **Dexamethasone**: 374,171 publications (187,658 general, 186,513 treatment)
2. **Metformin**: 231,379 publications (114,873 general, 116,506 treatment)
3. **Thalidomide**: 107,808 publications (49,513 general, 58,295 treatment)
4. **Valproate**: 101,890 publications (50,742 general, 51,148 treatment)
5. **Propranolol**: 71,534 publications (35,467 general, 36,067 treatment)
6. **Disulfiram**: 53,706 publications (27,062 general, 26,644 treatment)
7. **Ivermectin**: 48,482 publications (23,831 general, 24,651 treatment)
8. **Albendazole**: 44,341 publications (21,920 general, 22,421 treatment)
9. **Disulfiram (with Copper)**: 40,143 publications (19,782 general, 20,361 treatment)
10. **Mebendazole**: 39,224 publications (19,524 general, 19,700 treatment)

11. **Hydroxychloroquine (with Zinc)**: 39,158 publications (19,237 general, 19,921 treatment)
12. **Fenbendazole**: 35,930 publications (17,728 general, 18,202 treatment)

Key changes observed:

- **Dexamethasone** maintains the lead but with an 85.7% reduction (from 1,538,058 to 374,171)
- **Metformin** remains in second place, with a 66.8% reduction (from 697,172 to 231,379)
- The **extreme concentration** is significantly reduced
- The **top two medications** now represent approximately 53% of the total (vs. 70% previously)
- **Fenbendazole** continues to be the least represented, but the relative difference decreases considerably

New Herfindahl Index (without Google Scholar):

Adjusted Herfindahl Index: 0.1657

Comparison with the original index:

- Original Herfindahl Index: 0.2870 (Highly concentrated)
- Adjusted Herfindahl Index: 0.1657 (Moderately concentrated)
- Reduction: 42.2%

4. CONCLUSIONS

This bibliometric analysis quantified and mapped the research landscape for eleven off-patent medications being investigated for cancer treatment applications, revealing significant patterns in the allocation of scientific attention that have implications for future research prioritization.

Key findings demonstrate an extreme concentration of research, with dexamethasone and metformin collectively representing nearly 70% of all publications (69.28%), while the Herfindahl Index of 0.2870 confirms a highly concentrated research environment. This concentration pattern suggests that research attention may be disproportionately influenced by factors beyond anticancer efficacy alone, including clinical familiarity, funding priorities, and established therapeutic pathways.

An inverse relationship was identified between total publication volume and treatment specificity ratios. Medications with lower overall research attention, such as fenbendazole (87.82%), disulfiram with copper (86.54%), and hydroxychloroquine with zinc (75.21%), demonstrated higher proportions of cancer treatment-specific research compared to more established drugs. This pattern suggests that emerging candidates may generate more focused research specifically targeting cancer applications.

Pharmacological class analysis revealed varying research patterns. Antiparasitics (61.96%), antialcoholics (66.99%), and antimalarials (75.21%) exhibited high treatment specificity ratios, despite modest absolute volumes, indicating a focused interest in their anticancer mechanisms. Conversely, established classes like corticosteroids (18.71%) and antidiabetics (27.95%) demonstrated lower treatment specificity, suggesting broader research applications.

Database analysis showed that Google Scholar (64.39%) and Science.gov (33.63%) provided 98% coverage, while correlation patterns revealed differences in indexing approaches between databases, with implications for bibliographic research strategies in drug repurposing studies.

This bibliometric assessment offers insights into the distribution patterns of research across multiple off-patent anticancer candidates. The identified patterns may inform research prioritization decisions, highlighting medications with high treatment specificity ratios but modest absolute research volumes that warrant further investigation.

The findings suggest potential opportunities in less-studied candidates, particularly within classes showing high treatment specificity despite lower absolute volumes. The concentration patterns raise questions about resource allocation in drug repurposing research and suggest value in considering both established and emerging therapeutic candidates.

Further research combining bibliometric analysis with qualitative assessment of clinical significance could enhance the understanding of optimal research prioritization in drug repurposing. The methodological approach demonstrated here could be applied to other therapeutic areas or used to monitor evolving research trends over time.

5. DECLARATIONS

5.1. Study Limitations

Methodological Limitations: The study employs a purely quantitative analysis that records only total result numbers without categorizing by study type, such as *in vitro*, *in vivo*, or clinical trials. This approach does not evaluate the methodological quality or clinical relevance of studies and fails to distinguish between preliminary studies and advanced research. Additionally, the search strategy is limited to simple terms, such as "medication AND cancer," without employing synonyms, MeSH terms, or more sophisticated search strategies, which may have resulted in missing relevant studies that use different terminology.

Database Limitations: Significant heterogeneity exists among databases, as evidenced by the different indexing criteria used across platforms. The correlation between Google Scholar and PubMed is notably low, at 0.1562, indicating a possible uncontrolled overlap between the databases. Google Scholar's inclusion of grey literature may inflate results compared to more selective academic databases. Furthermore, the study does not specify the temporal period of searches, which may create a potential temporal coverage bias, as older medications may have had historical advantages, and changes in terminology over time are not considered.

Categorization Limitations: The pharmacological grouping approach is simplified and may not reflect specific anticancer mechanisms, particularly since some medications have multiple pharmacological actions. Combinations such as "disulfiram + copper" are treated separately, which may not accurately represent their therapeutic potential. The study lacks population and indication control, failing to distinguish between different cancer types, geographical variations in research, or trends by age group or specific populations.

Interpretative Limitations: The absence of qualitative analysis represents a significant limitation, as publication volume does not necessarily equate to clinical efficacy. The study does not evaluate the level of evidence of individual studies or consider the balance between negative and positive findings. There is also a lack of clinical contextualization, as the bibliometric findings are not related to regulatory approvals, the current clinical development status of medications, or the practical feasibility of repurposing efforts.

Technical Limitations: The presence of possible duplications poses a technical concern, as the same study may appear in multiple databases without adequate control for duplicate elimination. Preprints may be counted alongside published versions, potentially inflating certain medication counts. Additionally, language limitations may exist, as databases may have a bias toward English-language publications, resulting in the under-representation of literature in other languages.

These limitations collectively suggest that results should be interpreted as an initial panoramic view of the field, requiring complementary detailed studies to guide research and clinical development decisions. The findings provide valuable insights into research distribution patterns but should not be considered definitive evidence of therapeutic potential or research priority without additional qualitative assessment.

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5.4. Competing Interests

Declare any potential conflict of interest that exists in this publication.

5.6. AI use declaration

Artificial intelligence tools were utilized to support data analysis, statistical processing, and the textual structuring of this document. All results, interpretations, and conclusions were subsequently validated, reviewed, and refined by human researchers to ensure methodological accuracy and scientific rigor.

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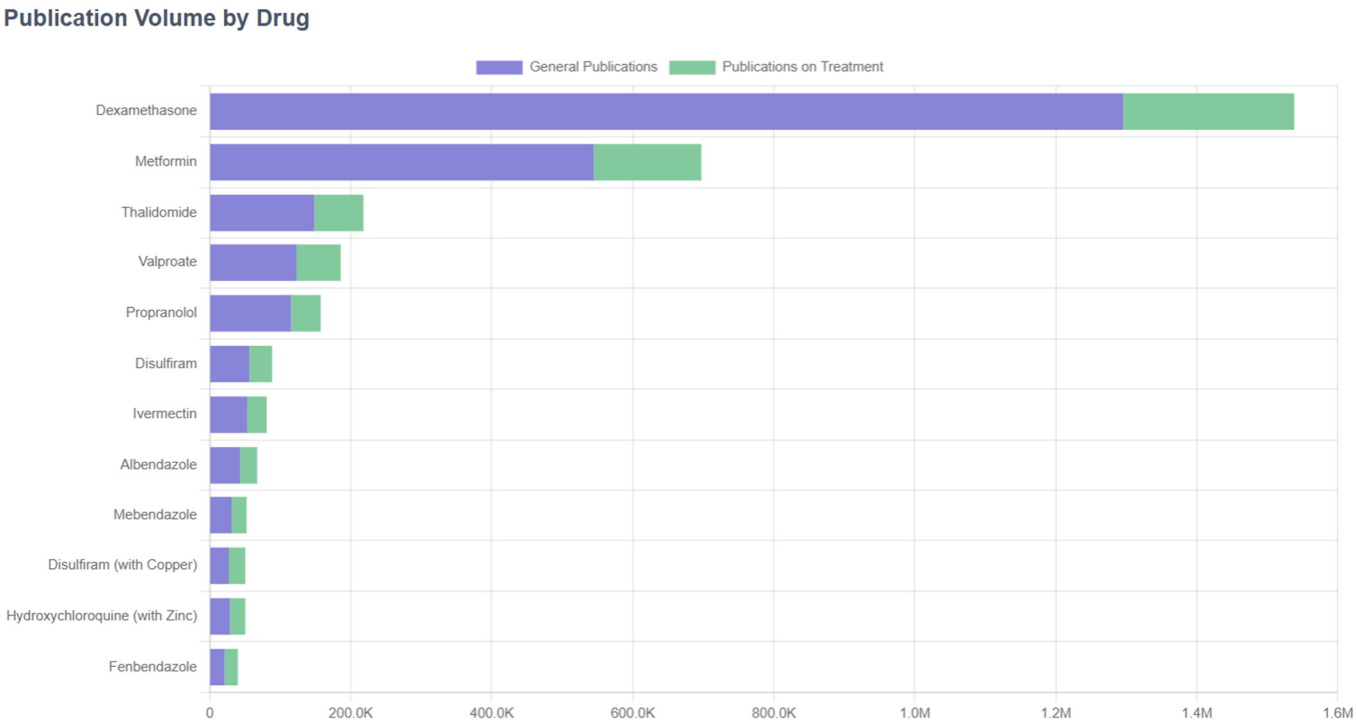

Figure 1. *Ranking of Drugs by Total Publication Volume*

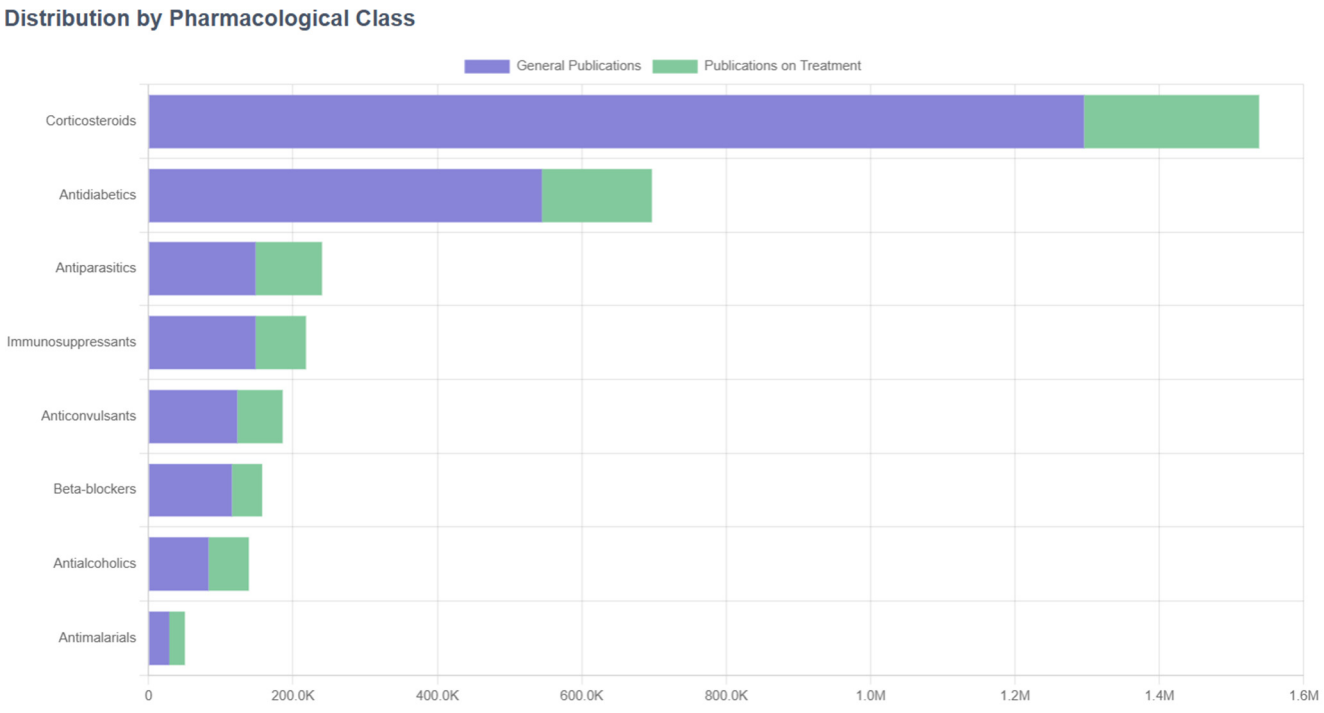

Figure 2. *Analysis by Pharmacological Classes*

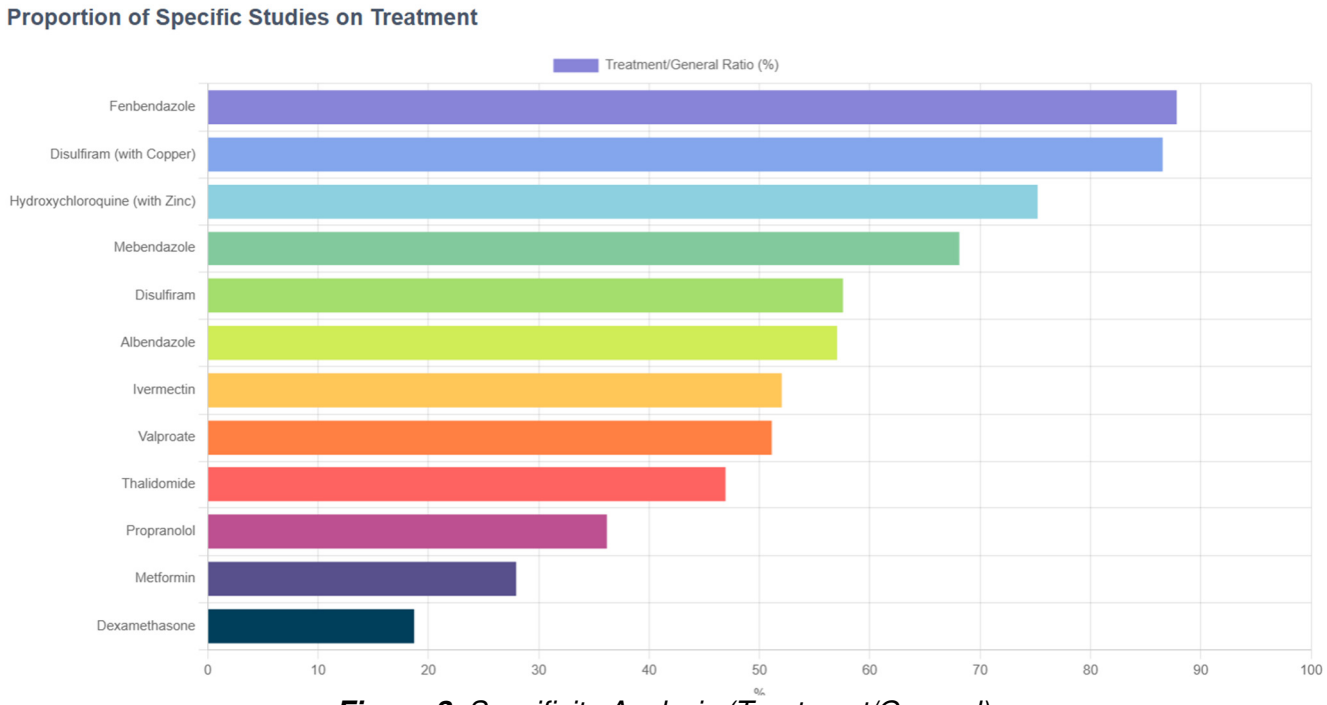

Figure 3. *Specificity Analysis (Treatment/General)*

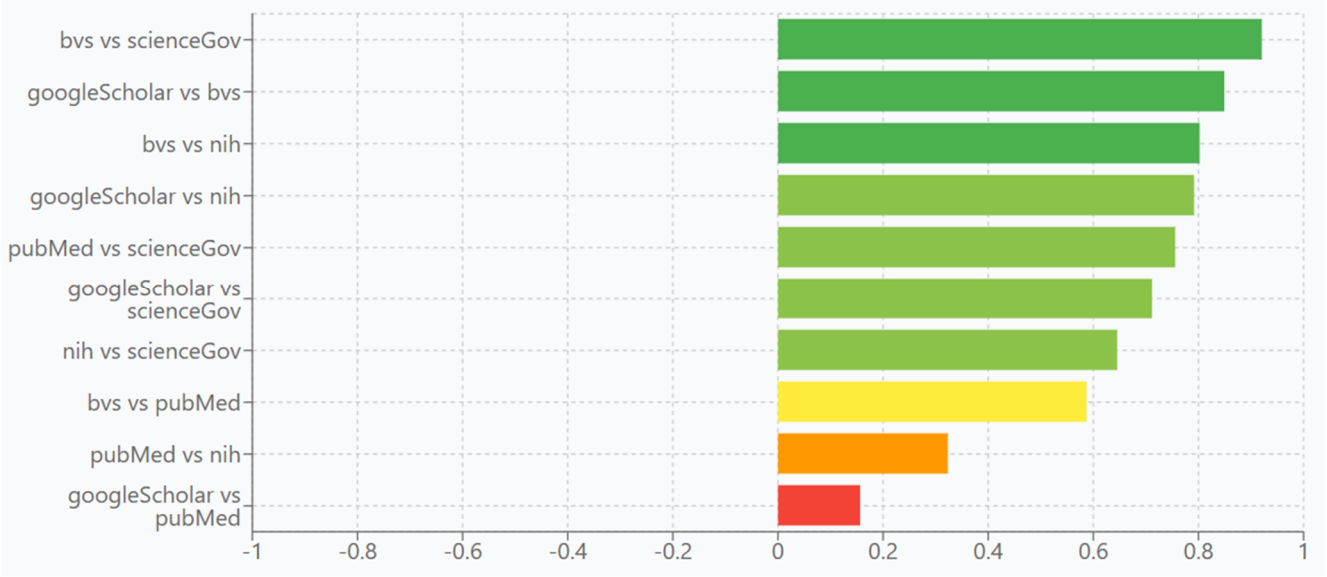

Figure 4. *Database Correlation Analysis.*

The correlation analysis examines the relationship between different databases in terms of result patterns. The high correlation suggests similar indexing or coverage patterns.

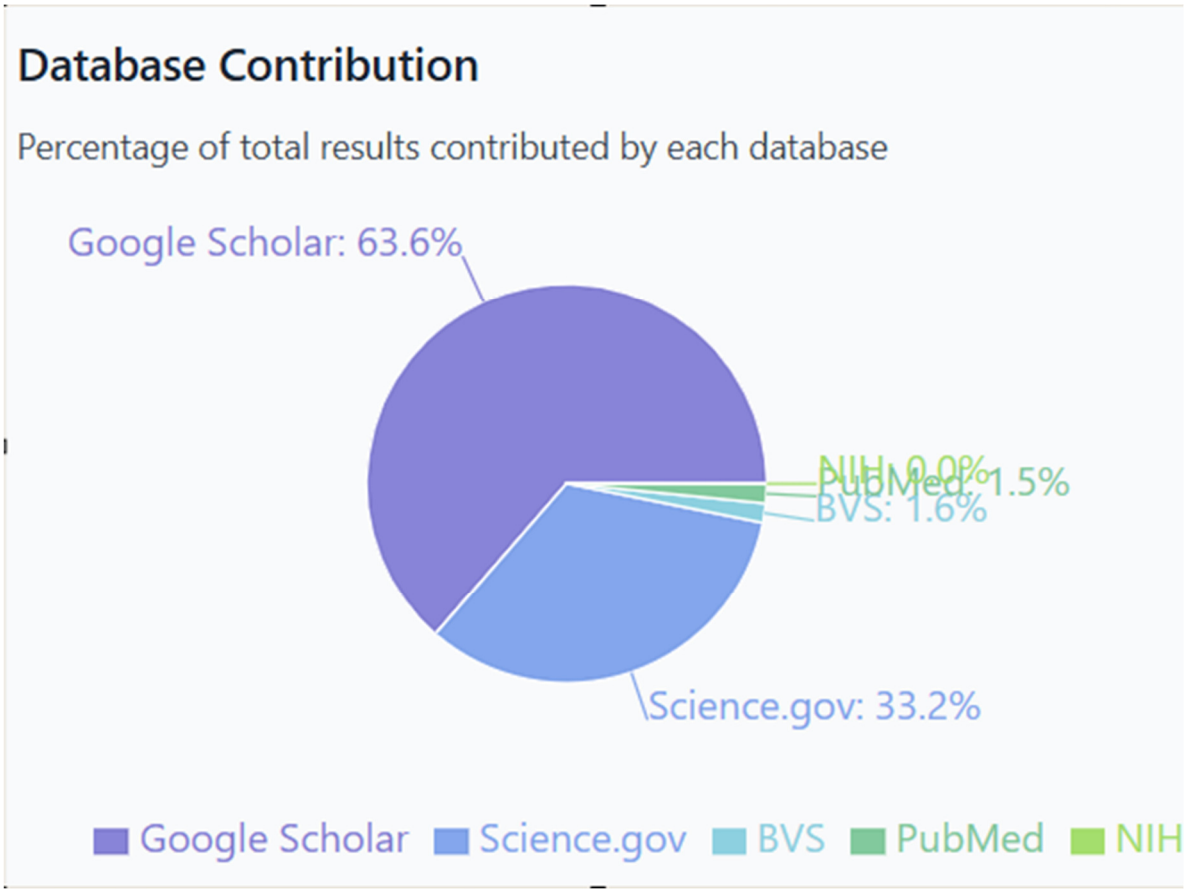

Figure 5. *Database Contribution*

Table 1. *Bibliometric search results for off-patent medications across five databases using cancer-related search terms*

Term	Google Scholar	BVS	PubMed	NIH	science.gov	Total
Ivermectin AND cancer	29.300	276	365	22	23168	53.131
Fenbendazole AND cancer	3.460	65	70	6	17587	21.188
Mebendazole AND cancer	11.600	193	230	10	19091	31.124
Albendazole AND cancer	21.000	287	421	10	21202	42.920
Metformin AND cancer	430.000	5.733	7,84	184	108948	544.873
Propranolol AND cancer	82.600	1.710	2,504	152	31101	115.566
Disulfiram AND cancer	29.100	762	802	68	25430	56.162
Valproate AND cancer	72.200	1.131	211	99	49301	122.942
Thalidomide AND cancer	101.000	5.147	2,102	188	42076	148.413
Dexamethasone AND cancer	1.110.000	15.546	1,994	262	169856	1.295.666
Hydroxychloroquine (with Zinc) AND cancer	9.510	8	7	20	19202	28.747
Disulfiram (with Copper) AND cancer	7.270	240	282	26	19234	27.052
Ivermectin AND cancer treatment	2.990	155	295	10	24191	27.641
Fenbendazole AND cancer treatment	405	33	55	0	18114	18.607
Mebendazole AND cancer treatment	1.500	172	21	0	19507	21.200
Albendazole AND cancer treatment	2.060	171	377	4	21869	24.481
Metformin AND cancer treatment	42.700	3.342	6,914	29	106221	152.299
Propranolol AND cancer treatment	7.790	1.111	2,054	24	32878	41.805
Disulfiram AND cancer treatment	5.690	439	638	18	25549	32.334
Valproate AND cancer treatment	13.500	756	1,805	28	48559	62.845
Thalidomide AND cancer treatment	17.500	3.951	6,155	67	48122	69.646
Dexamethasone AND cancer treatment	71.500	9.430	15,637	78	161368	242.392
Hydroxychloroquine (with Zinc) AND cancer treatment	1.700	6	6	1	19908	21.621
Disulfiram (with Copper) AND cancer treatment	3.050	157	262	10	19932	23.411

Anexo

CODE 1

```
<!DOCTYPE html>
<html lang="en">
<head>
    <meta charset="UTF-8">
    <meta name="viewport" content="width=device-width, initial-scale=1.0">
    <title>Bibliometric Analysis: Repurposed Drugs for Cancer</title>
    <style>
        body {
            font-family: Arial, sans-serif;
            line-height: 1.6;
            color: #333;
            max-width: 1200px;
            margin: 0 auto;
            padding: 20px;
        }
        h1 {
            color: #2c3e50;
            text-align: center;
            margin-bottom: 30px;
            border-bottom: 2px solid #3498db;
            padding-bottom: 10px;
        }
        h2 {
            color: #2980b9;
            margin-top: 30px;
            border-left: 4px solid #3498db;
            padding-left: 10px;
        }
        h3 {
            color: #3498db;
        }
        pre {
            background-color: #f8f9fa;
            border: 1px solid #ddd;
            border-radius: 4px;
            padding: 15px;
            overflow-x: auto;
            margin: 20px 0;
        }
        code {
            font-family: Consolas, Monaco, 'Andale Mono', monospace;
            color: #333;
        }
        .container {
            display: flex;
            flex-wrap: wrap;
            justify-content: space-between;
        }
        .column {
            flex: 0 0 48%;
            margin-bottom: 20px;
        }
        table {
            width: 100%;
            border-collapse: collapse;
            margin: 20px 0;
        }
        th, td {
            border: 1px solid #ddd;
```

```
            padding: 8px;
            text-align: left;
        }
        th {
            background-color: #f2f2f2;
        }
        tr:nth-child(even) {
            background-color: #f9f9f9;
        }
        .button {
            background-color: #3498db;
            color: white;
            padding: 10px 15px;
            border: none;
            border-radius: 4px;
            cursor: pointer;
            font-size: 16px;
        }
        .button:hover {
            background-color: #2980b9;
        }
        .results-container {
            background-color: #f8f9fa;
            border: 1px solid #ddd;
            border-radius: 4px;
            padding: 15px;
            margin-top: 20px;
        }
        #results {
            white-space: pre-wrap;
            font-family: Consolas, Monaco, 'Andale Mono', monospace;
            max-height: 500px;
            overflow-y: auto;
        }
    </style>
</head>
<body>
    <h1>Bibliometric Analysis: Repurposed Drugs for Cancer Treatment</h1>

    <div class="container">
        <div class="column">
            <h2>Summary</h2>
            <p>This page contains the complete code used to perform the bibliometric analysis of search results on repurposed drugs for cancer treatment. The goal is to enable research reproducibility and methodological transparency.</p>

            <h2>Instructions</h2>
            <p>This JavaScript code performs multiple statistical analyses on search data from scientific databases. To run the code:</p>
            <ol>
                <li>Copy the code from the "Complete Code" section</li>
                <li>Open your browser's console (F12 or Ctrl+Shift+J)</li>
                <li>Paste the code and press Enter</li>
                <li>Alternatively, click the "Run Analysis" button below</li>
            </ol>

            <button id="runButton" class="button">Run Analysis</button>
        </div>

        <div class="column">
            <h2>Input Data</h2>
```

```
            <p>The data used in this analysis are the search results from five databases (Google Scholar, BVS, PubMed, NIH, and Science.gov) for 12 drugs, each with two search variations:</p>
            <ul>
                <li>"drug AND cancer"</li>
                <li>"drug AND cancer treatment"</li>
            </ul>

            <p>The table below shows a sample of the data:</p>
            <table id="sampleTable">
                <thead>
                    <tr>
                        <th>Search</th>
                        <th>Google Scholar</th>
                        <th>BVS</th>
                        <th>PubMed</th>
                        <th>NIH</th>
                        <th>Science.gov</th>
                        <th>Total</th>
                    </tr>
                </thead>
                <tbody>
                    <!-- Will be filled by JavaScript -->
                </tbody>
            </table>
        </div>
    </div>

    <h2>Analysis Results</h2>
    <div class="results-container">
        <div id="results">Click "Run Analysis" to see the results.</div>
    </div>

    <script>
        // Structure of the bibliometric research data
        const data = [
            { drug: "Ivermectin AND cancer", googleScholar: 29300, bvs: 276, pubMed: 365, nih: 22, scienceGov: 23168, total: 53131 },
            { drug: "Fenbendazole AND cancer", googleScholar: 3460, bvs: 65, pubMed: 70, nih: 6, scienceGov: 17587, total: 21188 },
            { drug: "Mebendazole AND cancer", googleScholar: 11600, bvs: 193, pubMed: 230, nih: 10, scienceGov: 19091, total: 31124 },
            { drug: "Albendazole AND cancer", googleScholar: 21000, bvs: 287, pubMed: 421, nih: 10, scienceGov: 21202, total: 42920 },
            { drug: "Metformin AND cancer", googleScholar: 430000, bvs: 5733, pubMed: 7384, nih: 184, scienceGov: 101572, total: 544873 },
            { drug: "Propranolol AND cancer", googleScholar: 82600, bvs: 1710, pubMed: 2504, nih: 152, scienceGov: 31101, total: 115566 },
            { drug: "Disulfiram AND cancer", googleScholar: 29100, bvs: 762, pubMed: 802, nih: 68, scienceGov: 25430, total: 56162 },
            { drug: "Valproate AND cancer", googleScholar: 72200, bvs: 1131, pubMed: 211, nih: 99, scienceGov: 49301, total: 122942 },
            { drug: "Thalidomide AND cancer", googleScholar: 101000, bvs: 5147, pubMed: 2102, nih: 188, scienceGov: 42076, total: 148413 },
            { drug: "Dexamethasone AND cancer", googleScholar: 1110000, bvs: 15546, pubMed: 1994, nih: 262, scienceGov: 169856, total: 1295666 },
            { drug: "Hydroxychloroquine (with Zinc) AND cancer", googleScholar: 9510, bvs: 8, pubMed: 7, nih: 20, scienceGov: 19202, total: 28747 },
            { drug: "Disulfiram (with Copper) AND cancer", googleScholar: 7270, bvs: 240, pubMed: 282, nih: 26, scienceGov: 19234, total: 27052 },

            { drug: "Ivermectin AND cancer treatment", googleScholar: 2990, bvs: 155, pubMed: 295, nih: 10, scienceGov: 24191, total: 27641 },
```

```
    { drug: "Fenbendazole AND cancer treatment", googleScholar: 405, bvs: 33, pubMed: 55, nih: 0, scienceGov: 18114, total: 18607 },
    { drug: "Mebendazole AND cancer treatment", googleScholar: 1500, bvs: 172, pubMed: 21, nih: 0, scienceGov: 19507, total: 21200 },
    { drug: "Albendazole AND cancer treatment", googleScholar: 2060, bvs: 171, pubMed: 377, nih: 4, scienceGov: 21869, total: 24481 },
    { drug: "Metformin AND cancer treatment", googleScholar: 42700, bvs: 3342, pubMed: 6914, nih: 29, scienceGov: 106221, total: 152299 },
    { drug: "Propranolol AND cancer treatment", googleScholar: 7790, bvs: 1111, pubMed: 2054, nih: 24, scienceGov: 32878, total: 41805 },
    { drug: "Disulfiram AND cancer treatment", googleScholar: 5690, bvs: 439, pubMed: 638, nih: 18, scienceGov: 25549, total: 32334 },
    { drug: "Valproate AND cancer treatment", googleScholar: 13500, bvs: 756, pubMed: 1805, nih: 28, scienceGov: 48559, total: 62845 },
    { drug: "Thalidomide AND cancer treatment", googleScholar: 17500, bvs: 3951, pubMed: 6155, nih: 67, scienceGov: 48122, total: 69646 },
    { drug: "Dexamethasone AND cancer treatment", googleScholar: 71500, bvs: 9430, pubMed: 15637, nih: 78, scienceGov: 161368, total: 242392 },
    { drug: "Hydroxychloroquine (with Zinc) AND cancer treatment", googleScholar: 1700, bvs: 6, pubMed: 6, nih: 1, scienceGov: 19908, total: 21621 },
    { drug: "Disulfiram (with Copper) AND cancer treatment", googleScholar: 3050, bvs: 157, pubMed: 262, nih: 10, scienceGov: 19932, total: 23411 }
];

/**
 * Function that performs the complete analysis of bibliometric data
 * @return {string} Results formatted as text
 */
function runAnalysis() {
  let results = "";

  // Helper function to add text to results
  function appendResult(text) {
    results += text + "\n";
  }

  // Extract basic drug name
  const extractDrugName = (fullName) => {
    if (fullName.includes(" (with ")) {
      // For drugs with additions like "(with Zinc)"
      return fullName.split(" AND ")[0];
    } else {
      // For regular drugs
      return fullName.split(" AND ")[0];
    }
  };

  // Group by basic drug
  const drugMap = {};
  data.forEach(item => {
    const basicDrug = extractDrugName(item.drug);
    const isTreatment = item.drug.includes("treatment");

    if (!drugMap[basicDrug]) {
      drugMap[basicDrug] = {
        name: basicDrug,
        general: null,
        treatment: null
      };
    }

    if (isTreatment) {
```

```
      drugMap[basicDrug].treatment = item;
    } else {
      drugMap[basicDrug].general = item;
    }
  });

  const drugPairs = Object.values(drugMap);

  // 1. Basic Descriptive Analysis
  appendResult("1. BASIC DESCRIPTIVE ANALYSIS");
  appendResult("==========================");

  // Calculate basic statistics for total results
  const totalResults = data.map(item => item.total);
  const sum = totalResults.reduce((acc, val) => acc + val, 0);
  const mean = sum / totalResults.length;
  const sortedTotals = [...totalResults].sort((a, b) => a - b);
  const median = sortedTotals.length % 2 === 0
    ? (sortedTotals[sortedTotals.length / 2 - 1] + sortedTotals[sortedTotals.length / 2]) / 2
    : sortedTotals[Math.floor(sortedTotals.length / 2)];
  const variance = totalResults.reduce((acc, val) => acc + Math.pow(val - mean, 2), 0) /
totalResults.length;
  const stdDev = Math.sqrt(variance);

  appendResult(`Total number of entries: ${data.length}`);
  appendResult(`Total sum of results: ${sum}`);
  appendResult(`Average results per entry: ${mean.toFixed(2)}`);
  appendResult(`Median of results: ${median}`);
  appendResult(`Standard deviation: ${stdDev.toFixed(2)}`);
  appendResult(`Minimum value: ${Math.min(...totalResults)}`);
  appendResult(`Maximum value: ${Math.max(...totalResults)}`);
  appendResult("");

  // 2. Ranking of Drugs by Total Publication Volume
  appendResult("2. RANKING OF DRUGS BY TOTAL PUBLICATION VOLUME");
  appendResult("============================================================");

  // Sum total results for each basic drug (general + treatment)
  const drugTotals = drugPairs.map(pair => {
    const generalTotal = pair.general ? pair.general.total : 0;
    const treatmentTotal = pair.treatment ? pair.treatment.total : 0;
    return {
      drug: pair.name,
      generalTotal,
      treatmentTotal,
      combinedTotal: generalTotal + treatmentTotal
    };
  });

  // Sort by combined total
  drugTotals.sort((a, b) => b.combinedTotal - a.combinedTotal);

  // Display ranking
  appendResult("Ranking by total publication volume (general + treatment):");
  drugTotals.forEach((item, index) => {
    appendResult(`${index + 1}. ${item.drug}: ${item.combinedTotal} publications
(${item.generalTotal} general, ${item.treatmentTotal} treatment)`);
  });
  appendResult("");

  // 3. Analysis by Pharmacological Classes
  appendResult("3. ANALYSIS BY PHARMACOLOGICAL CLASSES");
```

```
appendResult("====================================");

// Define drug groups
const drugGroups = {
  "Antiparasitics": ["Ivermectin", "Fenbendazole", "Mebendazole", "Albendazole"],
  "Antidiabetics": ["Metformin"],
  "Beta-blockers": ["Propranolol"],
  "Antialcoholics": ["Disulfiram", "Disulfiram (with Copper)"],
  "Anticonvulsants": ["Valproate"],
  "Immunosuppressants": ["Thalidomide"],
  "Corticosteroids": ["Dexamethasone"],
  "Antimalarials": ["Hydroxychloroquine (with Zinc)"]
};

// Calculate total for each group
const groupTotals = {};
for (const [group, drugs] of Object.entries(drugGroups)) {
  groupTotals[group] = {
    generalTotal: 0,
    treatmentTotal: 0,
    combinedTotal: 0,
    drugs: drugs
  };

  drugs.forEach(drug => {
    const drugData = drugTotals.find(d => d.drug === drug);
    if (drugData) {
      groupTotals[group].generalTotal += drugData.generalTotal;
      groupTotals[group].treatmentTotal += drugData.treatmentTotal;
      groupTotals[group].combinedTotal += drugData.combinedTotal;
    }
  });
}

// Sort groups by total
const sortedGroups = Object.entries(groupTotals)
  .sort((a, b) => b[1].combinedTotal - a[1].combinedTotal);

// Display results by group
sortedGroups.forEach(([group, data]) => {
  appendResult(`Group: ${group}`);
  appendResult(`Total publications: ${data.combinedTotal}`);
  appendResult(`General publications: ${data.generalTotal}`);
  appendResult(`Treatment publications: ${data.treatmentTotal}`);
  appendResult(`Treatment/general ratio: ${(data.treatmentTotal / data.generalTotal *
100).toFixed(2)}%`);
  appendResult(`Drugs in the group: ${data.drugs.join(", ")}`);
  appendResult("");
});

// 4. Specificity Analysis (Treatment/General)
appendResult("4. SPECIFICITY ANALYSIS (TREATMENT/GENERAL)");
appendResult("============================================");

// Calculate the proportion of treatment results relative to general results
const specificityAnalysis = drugPairs.map(pair => {
  const generalTotal = pair.general ? pair.general.total : 0;
  const treatmentTotal = pair.treatment ? pair.treatment.total : 0;
  const ratio = generalTotal > 0 ? (treatmentTotal / generalTotal) * 100 : 0;

  return {
    drug: pair.name,
```

```
      generalTotal,
      treatmentTotal,
      ratio
    };
  });

  // Sort by ratio
  specificityAnalysis.sort((a, b) => b.ratio - a.ratio);

  // Display results
  appendResult("Proportion of 'treatment' results relative to 'general' results:");
  specificityAnalysis.forEach(item => {
    appendResult(`${item.drug}: ${item.ratio.toFixed(2)}% (${item.treatmentTotal} treatment /
${item.generalTotal} general)`);
  });
  appendResult("");

  // 5. Correlation Analysis Between Databases
  appendResult("5. CORRELATION ANALYSIS BETWEEN DATABASES");
  appendResult("==========================================");

  // Function to calculate Pearson correlation coefficient
  function calculateCorrelation(x, y) {
    const n = x.length;
    let sumX = 0;
    let sumY = 0;
    let sumXY = 0;
    let sumX2 = 0;
    let sumY2 = 0;

    for (let i = 0; i < n; i++) {
      sumX += x[i];
      sumY += y[i];
      sumXY += x[i] * y[i];
      sumX2 += x[i] * x[i];
      sumY2 += y[i] * y[i];
    }

    const numerator = n * sumXY - sumX * sumY;
    const denominator = Math.sqrt((n * sumX2 - sumX * sumX) * (n * sumY2 - sumY * sumY));

    return denominator === 0 ? 0 : numerator / denominator;
  }

  // Extract data from each database
  const databases = ["googleScholar", "bvs", "pubMed", "nih", "scienceGov"];
  const databaseValues = {};
  databases.forEach(db => {
    databaseValues[db] = data.map(item => item[db]);
  });

  // Calculate correlations between pairs of databases
  const correlations = [];
  for (let i = 0; i < databases.length; i++) {
    for (let j = i + 1; j < databases.length; j++) {
      const corr = calculateCorrelation(databaseValues[databases[i]], databaseValues[databases[j]]);
      correlations.push({
        pair: `${databases[i]} vs ${databases[j]}`,
        correlation: corr
      });
    }
  }
```

```
// Sort by correlation strength
correlations.sort((a, b) => Math.abs(b.correlation) - Math.abs(a.correlation));

// Display correlations
appendResult("Correlations between databases (Pearson coefficient):");
correlations.forEach(item => {
  const strength = Math.abs(item.correlation) > 0.8 ? "Very strong" :
                   Math.abs(item.correlation) > 0.6 ? "Strong" :
                   Math.abs(item.correlation) > 0.4 ? "Moderate" :
                   Math.abs(item.correlation) > 0.2 ? "Weak" : "Very weak";

  appendResult(`${item.pair}: ${item.correlation.toFixed(4)} (${strength})`);
});
appendResult("");

// 6. Relative Contribution Analysis
appendResult("6. RELATIVE CONTRIBUTION ANALYSIS");
appendResult("==================================");

// Calculate the percentage contribution of each drug to the total publications
const totalPublications = drugTotals.reduce((sum, item) => sum + item.combinedTotal, 0);

appendResult("Percentage contribution of each drug to the total publications:");
drugTotals.forEach(item => {
  const percentage = (item.combinedTotal / totalPublications) * 100;
  appendResult(`${item.drug}: ${percentage.toFixed(2)}%`);
});
appendResult("");

// Calculate Herfindahl index
const herfindahlIndex = drugTotals.reduce((sum, item) => {
  const marketShare = item.combinedTotal / totalPublications;
  return sum + (marketShare * marketShare);
}, 0);

appendResult(`Herfindahl Index (research concentration): ${herfindahlIndex.toFixed(4)}`);
appendResult(`Interpretation: ${
  herfindahlIndex < 0.01 ? "Highly diversified" :
  herfindahlIndex < 0.15 ? "Not concentrated" :
  herfindahlIndex < 0.25 ? "Moderately concentrated" : "Highly concentrated"
}`);
appendResult("");

// 7. Search Efficiency by Database
appendResult("7. SEARCH EFFICIENCY BY DATABASE");
appendResult("=========================================");

// Calculate the percentage contribution of each database
const databaseContributions = {};
databases.forEach(db => {
  const total = data.reduce((sum, item) => sum + item[db], 0);
  databaseContributions[db] = {
    total,
    percentage: (total / sum) * 100
  };
});

// Sort databases by contribution
const sortedDatabases = Object.entries(databaseContributions)
  .sort((a, b) => b[1].total - a[1].total);
```

```
            appendResult("Contribution of each database to the total results:");
            sortedDatabases.forEach(([db, data]) => {
              appendResult(`${db}: ${data.total} results (${data.percentage.toFixed(2)}% of total)`);
            });

            return results;
        }

        // Function to fill the sample table
        function fillSampleTable() {
            const sampleData = [
                { drug: "Ivermectin AND cancer", googleScholar: 29300, bvs: 276, pubMed: 365, nih: 22,
scienceGov: 23168, total: 53131 },
                { drug: "Metformin AND cancer", googleScholar: 430000, bvs: 5733, pubMed: 7384, nih: 184,
scienceGov: 101572, total: 544873 },
                { drug: "Dexamethasone AND cancer", googleScholar: 1110000, bvs: 15546, pubMed: 1994,
nih: 262, scienceGov: 169856, total: 1295666 },
                { drug: "Ivermectin AND cancer treatment", googleScholar: 2990, bvs: 155, pubMed: 295, nih:
10, scienceGov: 24191, total: 27641 },
                { drug: "Thalidomide AND cancer treatment", googleScholar: 17500, bvs: 3951, pubMed:
6155, nih: 67, scienceGov: 48122, total: 69646 }
            ];

            const tbody = document.querySelector('#sampleTable tbody');

            sampleData.forEach(item => {
                const row = document.createElement('tr');

                const drugCell = document.createElement('td');
                drugCell.textContent = item.drug;
                row.appendChild(drugCell);

                const gsCell = document.createElement('td');
                gsCell.textContent = item.googleScholar.toLocaleString();
                row.appendChild(gsCell);

                const bvsCell = document.createElement('td');
                bvsCell.textContent = item.bvs.toLocaleString();
                row.appendChild(bvsCell);

                const pmCell = document.createElement('td');
                pmCell.textContent = item.pubMed.toLocaleString();
                row.appendChild(pmCell);

                const nihCell = document.createElement('td');
                nihCell.textContent = item.nih.toLocaleString();
                row.appendChild(nihCell);

                const sgCell = document.createElement('td');
                sgCell.textContent = item.scienceGov.toLocaleString();
                row.appendChild(sgCell);

                const totalCell = document.createElement('td');
                totalCell.textContent = item.total.toLocaleString();
                row.appendChild(totalCell);

                tbody.appendChild(row);
            });
        }

        // Initialize page when loaded
        document.addEventListener('DOMContentLoaded', function() {
```

```
            fillSampleTable();

            // Add event listener to run button
            document.getElementById('runButton').addEventListener('click', function() {
                try {
                    const results = runAnalysis();
                    document.getElementById('results').textContent = results;
                } catch (error) {
                    document.getElementById('results').textContent = 'Error running analysis: ' +
error.message;
                    console.error('Analysis error:', error);
                }
            });
        });
    </script>
</body>
</html>
```

Code 2

```
<!DOCTYPE html>
<html lang="en">
<head>
    <meta charset="UTF-8">
    <meta name="viewport" content="width=device-width, initial-scale=1.0">
    <title>Bibliometric Analysis: Repurposed Drugs for Cancer Treatment</title>
    <style>
        body {
            font-family: Arial, sans-serif;
            background-color: #f7fafc;
            padding: 20px;
        }
        .container {
            max-width: 1200px;
            margin: 0 auto;
            background-color: #fff;
            padding: 20px;
            border-radius: 8px;
            box-shadow: 0 2px 4px rgba(0, 0, 0, 0.1);
        }
```

```
        h1 {
            text-align: center;
            color: #1a202c;
            margin-bottom: 20px;
        }
        h2 {
            font-size: 1.5rem;
            font-weight: bold;
            color: #2d3748;
            margin-top: 30px;
            margin-bottom: 15px;
            border-bottom: 2px solid #4299e1;
            padding-bottom: 5px;
        }
        .visualization-card {
            background-color: #fff;
            border-radius: 8px;
            box-shadow: 0 2px 4px rgba(0, 0, 0, 0.1);
            padding: 15px;
            margin-bottom: 20px;
        }
        .visualization-card h3 {
            font-size: 1.25rem;
            font-weight: 600;
            color: #4a5568;
            margin-bottom: 15px;
        }
        .grid {
            display: grid;
            grid-template-columns: 1fr;
            gap: 20px;
        }
        @media (min-width: 768px) {
            .grid {
                grid-template-columns: 1fr 1fr;
            }
        }
        canvas {
            max-width: 100%;
            height: auto;
        }
        .chart-container {
            min-height: 400px;
        }
        p {
            font-size: 0.875rem;
            color: #718096;
            text-align: center;
            margin-top: 10px;
        }
    </style>
</head>
<body>
    <div class="container">
        <h1>Bibliometric Analysis: Repurposed Drugs for Cancer Treatment</h1>

        <h2>1. Ranking of Drugs by Total Publication Volume</h2>
        <div class="visualization-card">
            <h3>Publication Volume by Drug</h3>
            <div class="chart-container">
                <canvas id="drugTotalsChart"></canvas>
            </div>
```

```
        </div>

        <h2>2. Analysis by Pharmacological Classes</h2>
        <div class="visualization-card">
            <h3>Distribution by Pharmacological Class</h3>
            <div class="chart-container">
                <canvas id="pharmacologicalGroupsChart"></canvas>
            </div>
        </div>

        <div class="grid">
            <div class="visualization-card">
                <h3>Treatment/General Ratio by Pharmacological Class (%)</h3>
                <div class="chart-container">
                    <canvas id="groupRatioChart"></canvas>
                </div>
            </div>
            <div class="visualization-card">
                <h3>Relative Contribution of Databases</h3>
                <div class="chart-container">
                    <canvas id="databaseContributionsChart"></canvas>
                </div>
            </div>
        </div>

        <h2>3. Specificity Analysis (Treatment/General)</h2>
        <div class="visualization-card">
            <h3>Proportion of Specific Studies on Treatment</h3>
            <div class="chart-container">
                <canvas id="specificityChart"></canvas>
            </div>
        </div>

        <h2>4. Comparison between Total Volume and Specificity</h2>
        <div class="visualization-card">
            <h3>Relationship between Total Publication Volume and Specificity by Drug</h3>
            <div class="chart-container">
                <canvas id="radarChart"></canvas>
            </div>
            <p>This radar chart shows the relationship between the total volume of publications (on a
logarithmic scale) and the proportion of specific studies on treatment for each drug.</p>
        </div>
    </div>

    <script src="https://cdn.jsdelivr.net/npm/chart.js@4.4.0/dist/chart.umd.min.js"></script>
    <script>
        // Raw data
        const data = [
            { drug: "Ivermectin AND cancer", googleScholar: 29300, bvs: 276, pubMed: 365, nih: 22,
scienceGov: 23168, total: 53131 },
            { drug: "Fenbendazole AND cancer", googleScholar: 3460, bvs: 65, pubMed: 70, nih: 6,
scienceGov: 17587, total: 21188 },
            { drug: "Mebendazole AND cancer", googleScholar: 11600, bvs: 193, pubMed: 230, nih: 10,
scienceGov: 19091, total: 31124 },
            { drug: "Albendazole AND cancer", googleScholar: 21000, bvs: 287, pubMed: 421, nih: 10,
scienceGov: 21202, total: 42920 },
            { drug: "Metformin AND cancer", googleScholar: 430000, bvs: 5733, pubMed: 7384, nih: 184,
scienceGov: 101572, total: 544873 },
            { drug: "Propranolol AND cancer", googleScholar: 82600, bvs: 1710, pubMed: 2504, nih: 152,
scienceGov: 31101, total: 115566 },
            { drug: "Disulfiram AND cancer", googleScholar: 29100, bvs: 762, pubMed: 802, nih: 68,
scienceGov: 25430, total: 56162 },
```

```
        { drug: "Valproate AND cancer", googleScholar: 72200, bvs: 1131, pubMed: 211, nih: 99, scienceGov: 49301, total: 122942 },
        { drug: "Thalidomide AND cancer", googleScholar: 101000, bvs: 5147, pubMed: 2102, nih: 188, scienceGov: 42076, total: 148413 },
        { drug: "Dexamethasone AND cancer", googleScholar: 1110000, bvs: 15546, pubMed: 1994, nih: 262, scienceGov: 169856, total: 1295666 },
        { drug: "Hydroxychloroquine (with Zinc) AND cancer", googleScholar: 9510, bvs: 8, pubMed: 7, nih: 20, scienceGov: 19202, total: 28747 },
        { drug: "Disulfiram (with Copper) AND cancer", googleScholar: 7270, bvs: 240, pubMed: 282, nih: 26, scienceGov: 19234, total: 27052 },
        { drug: "Ivermectin AND cancer treatment", googleScholar: 2990, bvs: 155, pubMed: 295, nih: 10, scienceGov: 24191, total: 27641 },
        { drug: "Fenbendazole AND cancer treatment", googleScholar: 405, bvs: 33, pubMed: 55, nih: 0, scienceGov: 18114, total: 18607 },
        { drug: "Mebendazole AND cancer treatment", googleScholar: 1500, bvs: 172, pubMed: 21, nih: 0, scienceGov: 19507, total: 21200 },
        { drug: "Albendazole AND cancer treatment", googleScholar: 2060, bvs: 171, pubMed: 377, nih: 4, scienceGov: 21869, total: 24481 },
        { drug: "Metformin AND cancer treatment", googleScholar: 42700, bvs: 3342, pubMed: 6914, nih: 29, scienceGov: 106221, total: 152299 },
        { drug: "Propranolol AND cancer treatment", googleScholar: 7790, bvs: 1111, pubMed: 2054, nih: 24, scienceGov: 32878, total: 41805 },
        { drug: "Disulfiram AND cancer treatment", googleScholar: 5690, bvs: 439, pubMed: 638, nih: 18, scienceGov: 25549, total: 32334 },
        { drug: "Valproate AND cancer treatment", googleScholar: 13500, bvs: 756, pubMed: 1805, nih: 28, scienceGov: 48559, total: 62845 },
        { drug: "Thalidomide AND cancer treatment", googleScholar: 17500, bvs: 3951, pubMed: 6155, nih: 67, scienceGov: 48122, total: 69646 },
        { drug: "Dexamethasone AND cancer treatment", googleScholar: 71500, bvs: 9430, pubMed: 15637, nih: 78, scienceGov: 161368, total: 242392 },
        { drug: "Hydroxychloroquine (with Zinc) AND cancer treatment", googleScholar: 1700, bvs: 6, pubMed: 6, nih: 1, scienceGov: 19908, total: 21621 },
        { drug: "Disulfiram (with Copper) AND cancer treatment", googleScholar: 3050, bvs: 157, pubMed: 262, nih: 10, scienceGov: 19932, total: 23411 }
    ];

    // Colors for the charts
    const COLORS = [
        '#8884d8', '#83a6ed', '#8dd1e1', '#82ca9d', '#a4de6c',
        '#d0ed57', '#ffc658', '#ff8042', '#ff6361', '#bc5090',
        '#58508d', '#003f5c'
    ];

    // Function to format large numbers
    function formatNumber(num) {
        if (num >= 1000000) return (num / 1000000).toFixed(1) + 'M';
        if (num >= 1000) return (num / 1000).toFixed(1) + 'K';
        return num;
    }

    // Process the data
    function processData() {
        // Extract basic drug name
        const extractDrugName = (fullName) => {
            if (fullName.includes(" (with ")) return fullName.split(" AND ")[0];
            return fullName.split(" AND ")[0];
        };

        // Group by basic drug
        const drugMap = {};
        data.forEach(item => {
            const basicDrug = extractDrugName(item.drug);
```

```
    const isTreatment = item.drug.includes("treatment");
    if (!drugMap[basicDrug]) {
      drugMap[basicDrug] = { name: basicDrug, general: null, treatment: null };
    }
    if (isTreatment) drugMap[basicDrug].treatment = item;
    else drugMap[basicDrug].general = item;
  });

  const drugPairs = Object.values(drugMap);

  // 1. Ranking of Drugs
  const drugTotals = drugPairs.map(pair => {
    const generalTotal = pair.general ? pair.general.total : 0;
    const treatmentTotal = pair.treatment ? pair.treatment.total : 0;
    return {
      drug: pair.name,
      generalTotal,
      treatmentTotal,
      combinedTotal: generalTotal + treatmentTotal
    };
  }).sort((a, b) => b.combinedTotal - a.combinedTotal).slice(0, 12);

  // 2. Pharmacological Classes
  const drugGroups = {
    "Antiparasitics": ["Ivermectin", "Fenbendazole", "Mebendazole", "Albendazole"],
    "Antidiabetics": ["Metformin"],
    "Beta-blockers": ["Propranolol"],
    "Antialcoholics": ["Disulfiram", "Disulfiram (with Copper)"],
    "Anticonvulsants": ["Valproate"],
    "Immunosuppressants": ["Thalidomide"],
    "Corticosteroids": ["Dexamethasone"],
    "Antimalarials": ["Hydroxychloroquine (with Zinc)"]
  };
  const pharmacologicalGroups = Object.entries(drugGroups).map(([group, drugs]) => {
    let generalTotal = 0, treatmentTotal = 0;
    drugs.forEach(drug => {
      const drugData = drugTotals.find(d => d.drug === drug) || { generalTotal: 0, treatmentTotal:
0 };

      generalTotal += drugData.generalTotal;
      treatmentTotal += drugData.treatmentTotal;
    });
    return {
      group,
      generalTotal,
      treatmentTotal,
      combinedTotal: generalTotal + treatmentTotal,
      ratio: generalTotal > 0 ? (treatmentTotal / generalTotal) * 100 : 0
    };
  }).sort((a, b) => b.combinedTotal - a.combinedTotal);

  // 3. Specificity
  const specificityData = drugPairs.map(pair => {
    const generalTotal = pair.general ? pair.general.total : 0;
    const treatmentTotal = pair.treatment ? pair.treatment.total : 0;
    return {
      drug: pair.name,
      generalTotal,
      treatmentTotal,
      ratio: generalTotal > 0 ? (treatmentTotal / generalTotal) * 100 : 0
    };
  }).sort((a, b) => b.ratio - a.ratio);
```

```
                // 4. Database Contributions
                const databases = ["googleScholar", "bvs", "pubMed", "nih", "scienceGov"];
                const databaseLabels = { googleScholar: "Google Scholar", bvs: "BVS", pubMed: "PubMed", nih:
"NIH", scienceGov: "Science.gov" };
                const databaseStats = {};
                let totalResults = 0;
                databases.forEach(db => {
                    const total = data.reduce((sum, item) => sum + item[db], 0);
                    totalResults += total;
                    databaseStats[db] = total;
                });
                const databaseContributions = databases.map(db => ({
                    name: databaseLabels[db],
                    value: databaseStats[db],
                    percentage: (databaseStats[db] / totalResults) * 100
                })).sort((a, b) => b.value - a.value);

                return { drugTotals, pharmacologicalGroups, specificityData, databaseContributions };
            }

            const { drugTotals, pharmacologicalGroups, specificityData, databaseContributions } =
processData();

            // Configure the charts
            new Chart(document.getElementById('drugTotalsChart'), {
                type: 'bar',
                data: {
                    labels: drugTotals.map(d => d.drug),
                    datasets: [
                        { label: 'General Publications', data: drugTotals.map(d => d.generalTotal),
backgroundColor: '#8884d8' },
                        { label: 'Publications on Treatment', data: drugTotals.map(d => d.treatmentTotal),
backgroundColor: '#82ca9d' }
                    ]
                },
                options: {
                    indexAxis: 'y',
                    scales: { x: { stacked: true, ticks: { callback: formatNumber } }, y: { stacked: true } },
                    plugins: { tooltip: { callbacks: { label: ctx => `${ctx.dataset.label}: ${new
Intl.NumberFormat().format(ctx.raw)}` } } }
                }
            });

            new Chart(document.getElementById('pharmacologicalGroupsChart'), {
                type: 'bar',
                data: {
                    labels: pharmacologicalGroups.map(g => g.group),
                    datasets: [
                        { label: 'General Publications', data: pharmacologicalGroups.map(g => g.generalTotal),
backgroundColor: '#8884d8' },
                        { label: 'Publications on Treatment', data: pharmacologicalGroups.map(g =>
g.treatmentTotal), backgroundColor: '#82ca9d' }
                    ]
                },
                options: {
                    indexAxis: 'y',
                    scales: { x: { stacked: true, ticks: { callback: formatNumber } }, y: { stacked: true } },
                    plugins: { tooltip: { callbacks: { label: ctx => `${ctx.dataset.label}: ${new
Intl.NumberFormat().format(ctx.raw)}` } } }
                }
            });
```

```
            new Chart(document.getElementById('groupRatioChart'), {
                type: 'bar',
                data: {
                    labels: pharmacologicalGroups.sort((a, b) => b.ratio - a.ratio).map(g => g.group),
                    datasets: [{
                        label: 'Treatment/General Ratio (%)',
                        data: pharmacologicalGroups.map(g => g.ratio),
                        backgroundColor: COLORS
                    }]
                },
                options: {
                    scales: { y: { beginAtZero: true, title: { display: true, text: 'Ratio (%)' } }, x: { ticks: { autoSkip:
false, maxRotation: 45, minRotation: 45 } } },
                    plugins: { tooltip: { callbacks: { label: ctx => `${ctx.dataset.label}: ${ctx.raw.toFixed(2)}%` } } }
                }
            });

            new Chart(document.getElementById('databaseContributionsChart'), {
                type: 'pie',
                data: {
                    labels: databaseContributions.map(d => d.name),
                    datasets: [{
                        data: databaseContributions.map(d => d.value),
                        backgroundColor: COLORS
                    }]
                },
                options: {
                    plugins: {
                        tooltip: { callbacks: { label: ctx => `${ctx.label}: ${new Intl.NumberFormat().format(ctx.raw)}
(${ctx.dataset.data[ctx.dataIndex].percentage.toFixed(1)}%)` } },
                        legend: { position: 'right' }
                    }
                }
            });

            new Chart(document.getElementById('specificityChart'), {
                type: 'bar',
                data: {
                    labels: specificityData.map(d => d.drug),
                    datasets: [{
                        label: 'Treatment/General Ratio (%)',
                        data: specificityData.map(d => d.ratio),
                        backgroundColor: COLORS
                    }]
                },
                options: {
                    indexAxis: 'y',
                    scales: { x: { max: 100, title: { display: true, text: '%' } } },
                    plugins: { tooltip: { callbacks: { label: ctx => `${ctx.dataset.label}: ${ctx.raw.toFixed(2)}%` } } }
                }
            });

            new Chart(document.getElementById('radarChart'), {
                type: 'radar',
                data: {
                    labels: specificityData.map(d => d.drug),
                    datasets: [
                        { label: 'Specificity (%)', data: specificityData.map(d => d.ratio), borderColor: '#8884d8',
backgroundColor: 'rgba(136, 132, 216, 0.6)' },
                        { label: 'Volume (log)', data: specificityData.map(d => Math.log10(drugTotals.find(dt =>
dt.drug === d.drug).combinedTotal) * 10), borderColor: '#82ca9d', backgroundColor: 'rgba(130, 202, 157, 0.6)' }
                    ]
```

```
                    },
                    options: {
                        scales: { r: { beginAtZero: true } },
                        plugins: { tooltip: { callbacks: { label: ctx => `${ctx.dataset.label}:
${ctx.raw.toFixed(2)}${ctx.dataset.label === 'Specificity (%)' ? '%' : ''}` } } }
                    }
                });
            </script>
        </body>
        </html>
```

SOUTHERN JOURNAL OF SCIENCES

ESTABLISHED IN 1993

Original research paper

DETECTION OF EPSTEIN-BARR VIRUS (EBV) IN WOMEN WITH BREAST CANCER IN IRAQ USING IN-SITU HYBRIDIZATION AND IMMUNOHISTOCHEMICAL TECHNIQUES

DETECÇÃO DO VÍRUS EPSTEIN-BARR (EBV) EM MULHERES COM CÂNCER DE MAMA NO IRAQUE UTILIZANDO TÉCNICAS DE HIBRIDIZAÇÃO IN-SITU E IMUNOHISTOQUÍMICA

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ABSTRACT

Background: The Epstein-Barr virus (EBV) has recently been identified in human breast cancer globally, potentially contributing to the initiation and progression of this malignancy, as well as gastric cancer, nasopharyngeal carcinoma, and bladder cancer. It has been newly associated with breast cancer. Globally, breast cancer affects more women than any other type of cancer. In Iraq, the prevalence of breast cancer is comparable. **Aims**: The study examined Iraqi women diagnosed with invasive ductal carcinoma (IDC) and invasive lobular carcinoma (ILC) to detect Epstein-Barr Virus Nuclear Antigen-1 (EBNA-1) and encoded RNA (EBER). **Methods**: A total of 50 formalin-fixed paraffin-embedded tissues from invasive ductal carcinoma (IDC) (92%) and invasive lobular carcinoma (ILC) (8%) biopsy samples constituted the case group, while 30 formalin-fixed paraffin-embedded tissues from non-cancerous breast tissue served as the control group. The presence of Epstein-Barr virus protein (EBER) in breast tissue was assessed using immunohistochemistry (IHC) and chromogenic in situ hybridization (CISH) methods. **Results**: EBER RNA signals were found in 31 (62%). EBER RNA signals were seen in 3 (10%) control group participants. Significant differences ($P<0.04$) were seen in EBV EBER RNA-positive signals among study groups. Immunohistochemistry showed nuclear brown staining in 34 (68%) breast cancer patients. Control group: 3 (10%). Discussion: The research identified a statistically significant correlation between EBV positivity and breast cancer among Iraqi women, especially concerning invasive ductal carcinoma. The results corroborate previous reports of elevated EBV levels in malignant breast tissues relative to controls. Although detection approaches such as CISH and IHC provide complementary insights, additional studies are needed. **Conclusions**: The study concludes that EBNA-1 and EBV EBER RNA were overexpressed in our population group.

Keywords: *Breast cancer, EBV DNA, CISH, IHC*

RESUMO

Introdução: O vírus Epstein-Barr (EBV) foi recentemente identificado em câncer de mama humano globalmente, potencialmente contribuindo para o início e progressão desta malignidade, bem como câncer

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gástrico, carcinoma nasofaríngeo e câncer de bexiga. Ele foi recentemente associado ao câncer de mama. Globalmente, o câncer de mama afeta mais mulheres do que qualquer outro tipo de câncer. No Iraque, a prevalência do câncer de mama é comparável. **Objetivos**: O objetivo do estudo examinou mulheres iraquianas diagnosticadas com carcinoma ductal invasivo (IDC) e carcinoma lobular invasivo (ILC) para detectar o Antígeno Nuclear 1 do Vírus Epstein-Barr (EBNA-1) e RNA codificado (EBER). Métodos: Um total de 50 tecidos fixados em formalina e embebidos em parafina de amostras de biópsia de carcinoma ductal invasivo (IDC) (92%) e carcinoma lobular invasivo (ILC) (8%) constituíram o grupo de casos, enquanto 30 tecidos fixados em formalina e embebidos em parafina de tecido mamário não canceroso serviram como grupo controle. A presença da proteína do vírus Epstein-Barr (EBER) no tecido mamário foi avaliada usando métodos de imunohistoquímica (IHC) e hibridização in situ cromogênica (CISH). **Resultados**: Sinais de RNA EBER foram encontrados em 31 (62%). Sinais de RNA EBER foram observados em 3 (10%) participantes do grupo controle. Diferenças significativas (P<0,04) foram observadas nos sinais positivos de RNA EBER do EBV entre os grupos de estudo. A imunohistoquímica mostrou coloração nuclear marrom em 34 (68%) pacientes com câncer de mama. Grupo controle: 3 (10%). **Discussão**: A pesquisa identificou uma correlação estatisticamente significativa entre a positividade para EBV e o câncer de mama entre mulheres iraquianas, especialmente em relação ao carcinoma ductal invasivo. Os resultados corroboram relatos anteriores de níveis elevados de EBV em tecidos mamários malignos em relação aos controles. Embora abordagens de detecção como CISH e IHC forneçam insights complementares, estudos adicionais são necessários. **Conclusões**: O estudo conclui que EBNA-1 e RNA EBER do EBV foram superexpressos em nosso grupo populacional.

Palavras-chave: *Câncer de mama, DNA do EBV, CISH, IHC*

1. INTRODUCTION

Globally, breast cancer affects more women than any other type of cancer (DeSantis *et al*., 2018). The situation is quite similar in Iraq, where breast cancer is similarly seen as a significant public health issue and the most common type of cancer (Hussain and Lafta,2021; Al Alwan,2022).

New evidence is connecting EBV infection to breast carcinogenesis, even though the exact causation of breast cancer is still uncertain and probably polygenic (Jin *et al*., 2020). Significant evidence suggests a link between EBV and an increased risk of breast cancer, which is an important finding. A statistically significant correlation was shown in one meta-analysis that was carried out (Jin *et al*., 2020). Even more so, EBV was more common in breast cancer tissues than in normal control tissues. There is a robust statistical connection, as shown by additional meta-analyses (Hsu *et al*., 2024).

Herpes simplex virus type 4, is more often known as the Epstein-Barr virus (EBV). This particular gamma-herpes virus is unique to humans. Animals do not serve as reservoirs. In 1964, Denis Burkitt identified and described the virus from lymphoma samples; two scientists, Anthony Epstein and Yvonne Barr, were responsible for the virus's naming (Jawetz *et al*., 2010).

Up to 95% of adults between the ages of 35 and 40 have been infected with EBV, and most people will contract the virus at some point in their lives (National Health Service, 2010). Numerous human neoplasms have been linked to EBV, the most common of which are endemic Burkitt lymphomas and undifferentiated nasopharyngeal carcinomas (NPCs). Other tumor types that have shown viral DNA include tonsil, salivary gland, and thymus carcinomas, as well as a small number of cases of malignancies affecting the female genital tract (Kutok and Wang,2006; Eligio *et al*., 2010).

One possible role for EBV in cervical tumor pathogenesis has been suggested. A hitherto unrecognized link between EBV and cervical squamous cell cancer. Several autoimmune diseases, including dermatomyositis, rheumatoid arthritis, Sjögren's syndrome, multiple sclerosis, and systemic lupus erythematosus, have been linked to viral infections in recent years (Toussirot and Roudier, 2008; Villegas *et al*., 2010; Lünemann, 2012).

EBV is involved in several aspects of host cell carcinogenesis, including immune system cells (lymphocyte) transformation. It can convert lymphocytes in vitro and is, hence, the most potent oncogenic virus (Thorley *et al*., 2008). note that the specific mechanisms behind B-cell transition remain unclear. Infected B lymphocytes are able to enter the cell cycle, continue to proliferate indefinitely, and avoid cell death because the virus uses CD21 and HLA class II molecules on their surface to do so (Klein *et al*., 2007). Even though no virus is being generated in these dormant cells, the EBV genome is being retained as a multicopy episome.

The Immunohistochemistry technique brings about several advantages. In contrast to

approaches that merely detect EBV DNA, this approach enables the direct detection of EBV proteins in tumor cells, specifically EBNA-1, and thus provides stronger evidence of an active EBV infection (Khabaz, 2013; Joshi *et al*., 2009). Additionally, IHC's standardized techniques guarantee consistency of data across different research, and its extensive availability in pathology labs corroborates its efficacy (Shahi *et al*., 2022).

At the level of the tumor microenvironment, IHC can detect the presence of the virus in certain cell types and also determine which cell types the virus targets. Additionally, the method can identify EBV across the whole tissue area (Khabaz, 2013; Joshi *et al*., 2009).

However, there are some shared considerations that we need to make. The method's potential for producing false-positive results due to non-specific responses against cellular proteins reduces its specificity for identifying EBV infections (Shahi *et al*., 2022). Another major drawback is that PCR can identify the entire EBV genome, whereas IHC can only detect EBV-specific proteins (Shahi *et al*., 2022). The proportion of tumor cells that test positive for EBV among malignant cells might vary greatly, ranging from 5% to 50%. When the EBV expression level is low among cases, this diversity of variation could lead to an underestimate (Khabaz *et al*., 2017).

When looking for Epstein-Barr virus in breast tumor tissues, chromogenic in situ hybridization stands out as a superior technique compared to the fluorescence in situ hybridization (FISH) assay. A chromogenic detection system is the main mechanism by which CISH operates. Because of this method, the specialized and costly fluorescence microscopy is unnecessary, and the examiner may see the results with a regular light microscope.

According to several studies (Isola and Tanner,2004; Jensen,2014; Rosa *et al*., 2013; Hanna and Kwok, 2006), CISH is the most practical and economical method for doing these types of tests in a laboratory setting, making it a great substitute for the highly specialized and costly FISH technique. As a second point, CISH's chromogenic signal is far more stable than FISH's fluorescent signal. This allows the material to be stored permanently for further testing or analysis. Then, the examiner can simultaneously observe tissue structure and EBV detection using CISH. Visible in the tissue, the chromogenic signal adds to our understanding of the tissues' histopathological state (Rosa *et al*., 2013).

1.1. Aim of study

1- Immunohistochemistry and the chromogenic in situ hybridization technique will be used to identify EBV DNA in breast malignant tissues and compare the results with those from normal breast tissues in order to evaluate any potential function of EBV in the progression of breast cancer.

2- Researching the age distribution of Iraqi women diagnosed with breast cancer and the factors that may influence their prognosis in order to develop more effective treatments and preventative measures.

3- Look into the possibility that the virus has a role in the onset or advancement of breast cancer.

4- To give a cytodiagnostic gold standard for comparison other than histology.

2. MATERIALS AND METHODS

Utilizing immunohistochemistry and in situ hybridization methods, this study assessed the frequency of EBV human herpes virus in breast cancer cases among Iraqi women residing in the southern region of the country. The research utilized eighty breast tissue blocks preserved in formalin and embedded in paraffin. Of these, forty-five blocks included breast carcinomas for the case study, and thirty blocks contained non-malignant breast tissues for the control. The blocks with better fixation and processing were used in the case study.

2.1. Materials

2.2.1. A - Malignant group (case of study):

The number of instances of breast cancer included in this study is fifty (50). The demographic parameters and patient ages were documented on the case sheets. Their ages varied from twenty-five to seventy-three.

2.2.2. B - Non-malignant group (control of study):

A total of thirty breast tissues, all of which were determined to be free of malignancy by pathology and preserved in paraffin, served as the control group. Ages varied from twenty-four to seventy-two.

From January 2022 to December 2023, all specimens, whether malignant or non-malignant, were included in the patient blocks. Maysan Governorate's Al-Sadr General Hospital and Central Public Health Laboratories were consulted for the selection of malignant and non-malignant blocks. Hematoxylin and eosin (H&E) stained tissue blocks were used to diagnose these patients based on pathological data found in laboratory files. We gathered information about patients' ages, dates of birth, tumor sizes, grades, and histological examinations by reviewing their pathological reports and re-reading their clinical records. The room temperature was used to store all samples.

2.2. Methods

2.2.1. Sample preparation:

The research and control groups' paraffin-embedded tissue blocks were gathered. Each of the blocks that had paraffin embedded was used to make new portions in the following ways: On unspecial slides, four (4) µm-thick slices were cut. Certified pathologists reviewed newly manufactured hematoxylin and eosin (H&E) stained slides and validated the diagnosis of cancer types and grades according to criteria given by the World Health Organization (WHO). All relevant histological slides were re-examined.

Two further sections, each 4 µm thick, were cut on positively charged slides in order to carry out the in-situ hybridization procedure and the Immunohistochemistry (IHC) technique, which are used to detect the presence of EBV DNA.

2.2.2. Staining procedure of IHC

The immunohistochemical analysis was performed using the ZytoChem Plus HRP Kit detection system. Following a 10-minute wash with buffer solution, slides were treated with 3% H_2O_2 solution, then incubated with blocking solution (protein block, Reagent 1) for 5 minutes.

Primary antibodies at optimal dilution (or negative control reagent) were applied for 30-60 minutes, followed by three 2-minute washes with buffer solution. The biotinylated secondary mouse antibody (Reagent 2) was then applied for 30-60 minutes. After three additional 2-minute washes, streptavidin-HRP-conjugate (Reagent 3) was used for 10-15 minutes, followed by three final 2-minute washes.

Visualization was achieved using a DAB solution (5-15 minutes) with the reaction terminated by distilled water. Mounting options included aqueous mounting with AEC or permanent mounting with either DAB or AEC.

For analysis, two independent pathologists examined each slide using a LEICA DM750 light microscope (Germany). Cell counting was performed at 400× magnification across 10 randomly selected fields. Nuclear brown staining in tumor cells was considered positive for the target protein.

2.2.3. In situ hybridization

Using chromogenic in situ hybridization (CISH), the ZytoFast EBV Probe may detect human Epstein-Barr virus (EBV) EBER RNA in formalin-fixed, paraffin-embedded specimens. This probe is designed to be used in conjunction with the ZytoVision Bremerhaven/Germany HRP-DAB, which is part of the ZytoFast PLUS CISH Implementation Kit High Sensitivity. There is only one size of the ZytoFast EBV, which is made up of Digoxigenin-labeled oligonucleotides (~ 0.2 ng/µl) that target mRNA sequences encoding EBER-1 and EBER-2 regions.

- We followed the instructions on the packaging for the ZytoVision DNA probe hybridization/Detection system in situ kit to make sure all of our chemicals were ready to use.
- To create 20x Wash Buffer TBS (WB5), follow the steps outlined in the assay protocol.
- No additional reagents in the kit are required for use. There is no need to dilute, mix, or reconstitute.
- The oven was used twice to sterilize the glassware, each time for four hours at 100 ˚C. The pipette tips, distilled water, and Eppendorf tubes were autoclaved for 20 minutes at 121 ˚C to ensure sterilization.
- Tissues known to contain the target marker were used to generate the positive tissue slide, which served as the control. For EBV, they accounted for nasopharyngeal carcinoma.
- In situ hybridization runs were accompanied by a negative control. With the exception of the diluted probe, all reagents were added to the negative control.

Epstein-Barr virus (EBV) EBER RNA is used as a radioactive molecular marker in in situ hybridization. With this hybridization/detection system used correctly, it will produce A nuclear staining pattern that indicates that the target cells

have a favorable reaction to Epstein-Barr virus (EBV) EBER RNA. When detected by horseradish peroxidase (HRP) and diaminobenzidine (DAB) at the particular position of the hybridization probe in positive test tissue, hybridized digoxigenin-labeled oligonucleotides emerge as a brown pattern using the ZytoFast PLUS CISH Implementation Kits. Infected cell nuclei were the primary locations of the positive hybridization signals.

3. RESULTS AND DISCUSSION

3.1. Results

Figure 1 illustrates the histopathological features of invasive ductal carcinoma (IDC) across three distinct differentiation grades. Moreover, the results for the same tissue with different Magnifications (4x,, and 40x) are displayed in supplementary figures for all grades (1S, 2S, 3S). The study analyzed 50 breast cancer cases, revealing that grade 2 (moderately differentiated) carcinoma predominated at 52% (26 cases), followed by grade 3 (poorly differentiated) at 30% (15 cases) and grade 1 (well-differentiated) at 18% (9 cases).

The hematoxylin and eosin (H&E) stained sections (400× magnification) demonstrate the progressive morphological alterations associated with increasing histological grade:

Panel A (Grade 1 IDC): This well-differentiated carcinoma exhibits tumor cells arranged in cohesive clusters with well-formed tubular structures. The neoplastic cells display minimal nuclear pleomorphism with relatively uniform, round-to-oval nuclei and inconspicuous nucleoli. Mitotic figures are rare, and the intervening stroma appears abundant. These features reflect a tumor that largely retains the architectural organization of normal breast tissue, correlating with less aggressive biological behavior and a more favorable prognosis.

Panel B (Grade 2 IDC): This moderately differentiated carcinoma demonstrates increased cellularity with moderate nuclear pleomorphism. Tubule formation is significantly reduced compared to grade 1, with tumor cells forming less organized glandular structures. Nuclear size and shape show greater variability, with more prominent nucleoli and occasional mitotic figures. The reduced stromal component and increased cellular density indicate intermediate differentiation and biological aggressiveness.

Panel C (Grade 3 IDC): This poorly differentiated carcinoma is characterized by sheets and nests of pleomorphic tumor cells with minimal to absent tubule formation. Nuclear features include marked variation in size and shape, prominent nucleoli, and coarse chromatin. Mitotic activity is typically elevated, and areas of comedo-type necrosis may be present. These histological features correspond to aggressive biological behavior and poorer clinical outcomes.

The observed distribution of histological grades (18% Grade 1, 52% Grade 2, and 30% Grade 3) aligns with the typical pattern reported in the literature for invasive ductal carcinoma, with a predominance of moderately differentiated tumors. This distribution has significant prognostic implications, as histological grade serves as an independent predictive factor for survival and therapeutic response.

The Nottingham Histologic Score (modified Scarff-Bloom-Richardson grading system) used in this assessment evaluates three morphological features: tubule/glandular formation, nuclear pleomorphism, and mitotic count. This standardized grading system remains fundamental in determining the biological behavior of breast carcinomas and guiding therapeutic decision-making.

3.1.1. Associations between breast carcinoma types & grades

In this study, fifty cases of breast carcinoma were examined. The invasive ductal carcinoma (IDC) was categorized according to tumor grades as follows: 7 (14%) were grade 1, 25 (50%) were grade 2, and 14 (28%) were grade 3. As shown in Figure 2, the invasive lobular carcinoma (ILC) cases were classified as follows: 2 (4%) were grade 1, 1 (2% for grade 2), and 1 (2% for grade 3). There were no discernible variations ($P>0.05$) in the types and grades of breast cancer.

3.1.2. Distribution of age group patients with breast carcinoma according to histological types

According to Figure 3, the histopathological analysis of breast carcinoma cases (N=50) demonstrated distinct patterns in the distribution of histological subtypes across different age cohorts. Invasive ductal carcinoma (IDC) was the predominant histological variant, accounting for 92% (n = 46) of all cases, while invasive lobular carcinoma (ILC) represented 8% (n = 4) of the study population.

Among IDC cases, a bimodal age distribution was observed with the highest incidence in the 40-49 year age group (n=14, 30.4% of IDC cases), followed by patients aged 60 years and above (n=13, 28.3%). The 50-59 year age group accounted for 12 cases (26.1%), while patients younger than 39 years represented the smallest proportion (n = 7, 15.2%). This distribution pattern aligns with epidemiological data reported in similar regional studies, though the relatively high proportion of patients under 50 years (45.6% of IDC cases) may reflect the younger age structure of the population or potential genetic and environmental factors specific to the study region.

The limited number of ILC cases (n=4) exhibited a notable concentration in the 40-49 year age group (n=3, 75% of ILC cases), with a single case reported in patients under 39 years (25%). Notably, no ILC cases were documented in patients aged 50 years or older. While this distribution pattern for ILC differs from typical Western data, where ILC is more frequently observed in older patients, the small sample size precludes definitive conclusions regarding age-specific ILC prevalence in this population.

Statistical analysis revealed no significant differences between histological subtypes across age groups ($p < 0.038$), suggesting that age alone may not be a determinative factor for developing specific histological variants of breast carcinoma in this cohort. However, the distinctive age distribution patterns observed warrant further investigation with larger sample sizes to elucidate potential age-related biological mechanisms underlying different histological presentations of breast carcinoma.

These findings may have implications for screening strategies and clinical management, particularly considering the substantial proportion of breast cancer cases occurring in younger women in this population compared to Western cohorts.

3.1.3. Distribution of age group patients with breast carcinoma according to histological grades

According to Figure 4, the analysis of breast carcinoma cases (N=50) reveals a distinct distribution pattern of histological grades across different age cohorts. Grade 2 (moderately differentiated) carcinoma emerged as the predominant histological classification, comprising 52% (n = 26) of all cases, followed by Grade 3 (poorly differentiated) at 30% (n = 15) and Grade 1 (well-differentiated) at 18% (n = 9).

The distribution of Grade 2 carcinoma demonstrated a notable concentration in the 40-49 year age group, with 11 cases (42.3% of all Grade 2 cases) occurring in this cohort. The remaining Grade 2 cases were distributed equally between the 50-59 and ≥ 60-year age groups (6 cases each, representing 23.1% of Grade 2 cases per group), while only 3 cases (11.5%) were observed in patients younger than 39 years.

Grade 3 (poorly differentiated) carcinoma exhibited a more uniform distribution across younger and middle-aged cohorts, with 4 cases each in the <39 and 40-49 year groups (26.7% of Grade 3 cases per group) and 5 cases (33.3%) in the 50-59 year group. Significantly, only 2 cases (13.3%) were identified in patients aged ≥60 years, suggesting a possible inverse relationship between age and tumor aggressiveness.

Grade 1 (well-differentiated) carcinoma exhibited a distinctive age-related pattern, with a marked predominance in the ≥60-year age group (5 cases, 55.6% of all Grade 1 cases). The remaining Grade 1 cases were distributed sparsely across other age groups, with 2 cases (22.2%) in the 40-49 year cohort and only 1 case (11.1%) in each of the <39 and 50-59 year groups.

Statistical analysis revealed no significant differences in the distribution of histological grades across age groups ($p = 0.057$), although the p-value approached the threshold for statistical significance. This near-significant trend suggests potential age-related biological differences in breast carcinogenesis that may be confirmed statistically with a larger sample size.

The observed distribution patterns align with established literature, suggesting that well-differentiated tumors tend to occur more frequently in older patients, while poorly differentiated, more aggressive tumors are relatively more common in younger age groups. These findings may have implications for age-specific screening protocols and therapeutic approaches, particularly in populations with demographic and genetic characteristics similar to those of our study cohort.

3.1.4. Evaluation of EBV signals between study groups

According to Table 1, Epstein-Barr virus (EBV) detection rates in breast tissue samples showed marked differences between case and

control groups, as determined by both chromogenic in situ hybridization (CISH) and immunohistochemistry (IHC) techniques. The study encompassed 50 breast carcinoma cases and 30 control samples from non-malignant breast tissue.

The CISH method, which detects EBV-encoded RNA (EBER), revealed positive signals in 31 of 50 breast carcinoma cases (62%), characterized by brown discoloration at the site of complementary sequences in the nucleus of infected cells. In contrast, only 3 of 30 control samples (10%) exhibited positive EBER signals. The difference in EBV detection between case and control groups using CISH was statistically significant ($p<0.004$), suggesting a strong association between EBV presence and breast malignancy.

Similarly, IHC analysis demonstrated positive EBV signals in 34 of 50 breast carcinoma cases (68%), evidenced by nuclear brown staining of tumor cells using horseradish peroxidase (HRP) detection with DAB chromogen. Among the control group, only 3 of 30 samples (10%) showed positive IHC signals. This difference was also statistically significant ($p < 0.003$), further corroborating the association between EBV and breast carcinoma.

The data reveal a high level of concordance between CISH and IHC methods, with marginally higher detection rates using IHC (68%) compared to CISH (62%) in breast carcinoma cases. This slight difference could be attributed to the distinct molecular targets of each technique—CISH detects viral RNA while IHC identifies viral proteins, potentially capturing different stages of viral activity within tumor cells.

The highly significant p-values ($p < 0.004$ for CISH and $p < 0.003$ for IHC) strongly support the hypothesis that EBV infection is associated with breast carcinogenesis. The substantially higher detection rates in malignant tissue (62-68%) compared to non-malignant control tissue (10%) suggest that EBV may play a role in breast cancer pathogenesis rather than representing incidental colonization.

These findings align with emerging evidence implicating EBV in breast carcinogenesis, though the precise mechanisms through which the virus may contribute to malignant transformation remain to be fully elucidated. The dual-method approach employed in this study provides robust technical validation for the observed association between EBV and breast carcinoma.

3.2. Discussions

Globally, breast cancer affects more women than any other type of cancer (DeSantis *et al*., 2018). The situation is similar in Iraq, where breast cancer represents a significant public health issue and remains the most common malignancy (Hussain and Lafta,2021; Al Alwan,2022).

Emerging evidence connects Epstein-Barr virus (EBV) infection to breast carcinogenesis, although the exact etiology of breast cancer remains uncertain and is likely polygenic (Jin *et al*., 2020). Several studies suggest a significant link between EBV and increased breast cancer risk. A meta-analysis demonstrated a statistically significant correlation between EBV infection and breast cancer development (Jin *et al*., 2020). Furthermore, EBV prevalence was higher in breast cancer tissues compared to normal control tissues, with additional meta-analyses confirming this robust statistical association (Hsu *et al*., 2024).

However, the literature presents some discrepancies. Certain studies failed to establish a connection between EBV and the clinicopathological features of breast cancer tumors (Salih *et al*., 2022). Some researchers suggest that negative EBV findings in breast cancer tissues by immunohistochemistry (IHC) might result from limitations in detection techniques rather than the actual absence of the virus (Deshpande *et al*., 2002).

These conflicting results highlight the necessity for accurate EBV detection in breast cancer patients. The methodology employed in our study is particularly significant as it combines polymerase chain reaction (PCR) and chromogenic in situ hybridization (CISH) with immunohistochemistry (De *et al*., 2022; Gutjahr *et al*., 2023). EBER-CISH (EBV-encoded RNA chromogenic in situ hybridization) is considered the gold standard for identifying EBV infection in cancerous tissues due to its precise localization capabilities and high sensitivity (Luk *et al*., 2019).

This case-control study, therefore, aims to investigate analytical indicators of Epstein-Barr virus infection in breast tumors, contributing to the ongoing research exploring the potential association between EBV and breast carcinogenesis.

3.2.1. Histological types & grades of breast carcinoma cases

In this study, invasive ductal carcinoma (IDC) accounted for 92% of cases (46/50), while invasive lobular carcinoma (ILC) represented 8% (4/50). Analysis of histological grades revealed that grade 2 tumors predominated in 52% of cases, followed by grade 3 (30%) and grade 1 (18%), as illustrated in Figures 4-2 and 4-3.

Among IDC cases, the distribution by grade was: grade 1 in 7 patients (14%), grade 2 in 25 patients (50%), and grade 3 in 14 patients (28%). ILC was relatively uncommon, with only 4 cases: 2 cases of grade 1 (4%), 1 case of grade 2 (2%), and 1 case of grade 3 (2%), as shown in Figure 4-5. Statistical analysis revealed no significant difference between grades and histological subtypes ($p > 0.05$).

Our findings align with current literature indicating that IDC is the most common breast cancer subtype, typically presenting as grade 2, followed by grade 3 and grade 1 [27,28]. Previous studies report that IDC accounts for 75-80% of breast cancer cases, while ILC represents 10-15% (Howlader *et al*., 2020; Weigelt *et al*., 2010). The distribution pattern of IDC by tumor grade in our study confirms the pattern initially described by Elston and Ellis in 1991, with a predominance of grade 2 followed by grades 3 and 1.

Regarding ILC, the literature indicates that most tumors are of lower grade, with 60-80% classified as grade 1 or 2 (Li *et al*., 2005). Comparative studies between ILC and IDC have consistently shown that ILC generally exhibits a lower grade distribution. Denkert et al. characterized ILC as a "luminal A breast cancer prototype," typically presenting as a low-grade malignancy. However, Boughey et al. (2016) (Boughey and Nguyen, 2016) documented higher-grade HER2-positive ILC associated with more aggressive disease progression, while another study identified higher tumor grades in a specific ILC subtype compared to IDC (Mittendorf *et al*., 2014).

The predominance of grade 2 IDC in our cohort reflects the typical pattern described in the literature, with grade 1 being less frequent and grade 3 often observed in younger patients or those with more advanced diseases (Van *et al*., 2022). While both IDC and ILC can be classified as grade 3, the invasive nature and higher prevalence of IDC increase the likelihood of a grade 3 diagnosis in this subtype (Haagensen *et al*., 1978). Multiple studies support this observation, noting that higher-grade IDC constitutes a significant proportion of breast cancer cases (Rakha *et al*., 2010).

Our study's findings regarding the distribution of histological grades in IDC and the lower incidence of ILC are consistent with previous breast carcinoma research (Van *et al*., 2022; Jaroensri *et al*., 2022). The lower-than-expected prevalence of ILC in our cohort aligns with findings from other studies examining this less common breast cancer subtype.

3.2.2. Interpretations of the results of age group patients with breast carcinoma according to histological types

Our study revealed that invasive ductal carcinoma (IDC) was the predominant histological subtype, accounting for 92% ($n = 46$) of all cases. Among IDC patients, the 40-49 age group was most affected, with 14 cases, followed by the 60+ age group, with 13 cases. Invasive lobular carcinoma (ILC) accounted for only 8% ($n=4$) of cases, with the majority occurring in the 40-49 age bracket, as illustrated in Figure 4-8. Statistical analysis revealed no significant differences in outcomes between age groups or breast cancer subtypes ($p > 0.038$).

The incidence of invasive ductal carcinoma demonstrates considerable variation across different populations and geographical regions. A Nigerian study of 81 cases reported a mean diagnosis age of 45.06 years, with women aged 30-39 comprising approximately 38.3% of subjects (Ebughe *et al*. 2013). In contrast, a larger cohort study involving 279 breast cancer patients reported a wider age range with a mean age of 57 years (Tbeileh *et al*., 2024).

Regional disparities in IDC diagnosis age are notable. In Saudi Arabia, the median detection age is 54 years (mean, 55.68 years), with 61.7% of patients diagnosed before the age of 50 (Alabdulkarim *et al*., 2018). By comparison, the average diagnosis age in the United States and United Kingdom is 62 years, with a disproportionately high incidence among women aged 70 and older (DeSantis *et al*., 2018; Asiri *et al*., 2020). These variations highlight the influence of cultural, environmental, and geographical factors on IDC epidemiology, underscoring the need for early detection and sex-specific screening programs, particularly in regions where IDC prevalence is higher among younger women (Ebughe *et al*. 2013).

Invasive lobular carcinoma (ILC) typically

affects older women, though exceptions exist. A Nigerian study examining breast cancer cases (including ILC) reported an age range of 21-79 years, with a mean diagnosis age of 42.0 years (Ibrahim *et al*., 2015). Another study found a stronger correlation between mammographic density and ER-negative breast cancer (including ILC) in younger women (<55 years) compared to older women (Bertrand *et al*., 2013).

A comprehensive meta-analysis of 40 studies involving 87,303 patients reported a mean age of 54.9 years for ILC compared to 50.9 years for IDC (Jung *et al*., 2010). Further research indicates that patients diagnosed at or below this age group demonstrate lower overall survival rates compared to those in middle-aged categories (Liu *et al*., 2016). The increased prevalence of ILC among women over 50 years warrants additional investigation to inform appropriate preventive measures (Yang *et al*., 2020).

The age distribution patterns observed in our study and the reviewed literature suggest the need for age-appropriate screening strategies that account for the varying presentation of breast cancer subtypes. The younger presentation of IDC in certain populations underscores the importance of early detection programs, while the typically later onset of ILC highlights the continued need for screening in older age groups.

3.2.3. Interpretations of the results of age group patients with breast carcinoma according to histological grades

Another objective of our study was to look at the age distribution of breast cancer patients by histological grade. The subtype of breast cancer most commonly found in women aged 40–49 was grade 2, which accounted for 26 instances (52%). The age group most impacted was those between 50 and 59 years old, accounting for 30% of the total cases (15 diagnoses). Figure (4–9) shows that 9 out of the 100 participants (18%) had grade 1 carcinoma, with the majority of these cases being older adults (>60 years). A statistical analysis revealed no correlation ($p = 0.057$) between age group and breast cancer grade. The current study's findings support those of Nixon et al. (1994), which indicate that people aged 40 to 49 years old had a higher rate of grade 2 tumors, while those aged 50 to 59 years old had a higher rate of grade 3 tumors. On the other hand, it argues that age does not significantly impact cancer grade outcomes, which goes against our assertion that there is no robust correlation between the two. At the same time, we found an increased incidence of grade 3 tumors in older age groups (Alabdulkarim *et al*., 2018). However, showing the age and grade as having a complicated connection contradicts our conclusion that there is no major association. This study lends credence to the idea that age and cancer grade should not be considered together when making decisions, likewise suggests that there is no positive correlation between the two variables, but rather that age and breast cancer outcomes are correlated in a U-shaped fashion (Xie *et al*., 2023). Grade 2 tumors in younger girls and grade 3 tumors in older females are similar (Koshariya *et al*., 2022). Regarding the prevalence of grade 3 tumors in the 50-59 year old age group (Chen *et al*., 2016). Potential association between the higher-grade malignancies in senior females and similar data regarding the occurrence of grade 2 tumors in young females (Feizi *et al*., 2023). Despite our claims to the contrary, we found no correlation between age and cancer grade (Kroman *et al*., 2000). Aggressive tumor features and a poor prognosis in patients can be independently correlated with age, according to them. Contrarily, our earlier findings are somewhat contradicted, which state that younger patients exhibit more aggressive tumor subtypes and poorer overall survival. Our results show that older patients had a higher proportion of grade 3 tumors; however, this finding is expanded upon by suggesting that cultural and demographic variables may moderate the association between age, tumor grade, and survival (Alabdulkarim *et al*., 2018). Lastly, cast doubt on our results about the frequency of grade 3 tumors in older women, highlighting the fact that younger age is better associated with a worse prognosis and greater tumor grade (Cai *et al*., 2020).

3.2.4. Interpretation of EBV positivity among breast cancer cases and controls

As shown in Table 1 of our investigation, we compared 50 samples of breast cancer malignancy with non-malignant control tissues to determine the prevalence of EBV positivity by immunohistochemistry and chromogenic in situ hybridization. In a study involving 50 cases, 68% of the patients were found to have EBV antibodies detected by IHC and 62% by CISH. In comparison, 10% of the 30 control tissue samples tested positive for EBV antibodies by IHC or CISH. The statistical test findings for both approaches were significant, with a P-value less than 0.004.

There is a robust direct correlation between the EBV positivity status and the existence or absence of breast cancer, as shown by the statistically significant test findings. Although the precise percentages of EBV positivity differ, our study's results are consistent with others that have reached similar conclusions. Our results were consistent with those of Hussein (2013), who also found a statistically significant correlation between EBV-positive patients and controls. Also, a greater percentage of breast cancer patients were EBV-positive compared to healthy controls, suggesting a strong correlation between EBV infection and breast cancer (Mezher *et al*., 2013). A statistically significant correlation was found between IDC cases and EBV positivity, with a larger proportion of tumors displaying this status (Abd *et al*., 2021).

Research on EBV in Egyptian and Iraqi females with primary invasive breast cancer (PIBC) has shown promising results. In their study, they found that EBV was more prevalent in PIBC patients than in the control group (Zekri *et al*., 2012). Specifically, 45% of Egyptians and 28% of Iraqis tested positive for EBV, while 0% of the control group tested negative ($p < 0.05$). The distribution of many viruses, including EBV, in breast cancer tissues of Egyptian patients was examined in a study by Metwally et al. (2021). In contrast to normal breast tissue, 49% of breast cancer cases tested positive for EBV. The link between EBV positive and clinical outcomes of breast cancer patients was not, however, shown in this investigation.

Although the precise percentages of EBV positivity differ, our study's results are consistent with those of others that have reached similar conclusions and discovered a statistically significant correlation between EBV-positive patients and controls (Hussein, 2013). Also, a greater percentage of breast cancer patients were EBV-positive compared to healthy controls, suggesting a strong correlation between EBV infection and breast cancer (Mezher *et al*., 2013). A statistically significant correlation was found between IDC cases and EBV positivity, with a larger proportion of tumors displaying this status, according to research (Abd *et al*., 2021).

Research on EBV in Egyptian and Iraqi females with primary invasive breast cancer (PIBC) has shown promising results. EBV was more prevalent in PIBC patients than in the control group. Specifically, 45% of Egyptians and 28% of Iraqis tested positive for EBV, while 0% of the control group tested negative ($p < 0.05$) [55]. The distribution of many viruses, including EBV, in breast cancer tissues of Egyptian patients was examined [56]. In contrast to normal breast tissue, 49% of breast cancer cases tested positive for EBV. The link between EBV positive and clinical outcomes of breast cancer patients was not, however, shown in this investigation.

4. CONCLUSIONS

Recent data indicate that the Epstein-Barr virus may be involved in the development of breast cancer, particularly in aggressive forms of the disease. Our study revealed significantly higher EBV expression in breast cancer tissues compared to control specimens using both IHC and CISH methodologies, consistent with previous research demonstrating increased prevalence of EBV in malignant breast tissues. The results of the present study clearly establish that EBV was more frequently expressed in breast cancer tissues than in the control group, regardless of whether IHC or CISH evaluation methods were employed.

Both immunohistochemistry (IHC) and chromogenic in situ hybridization (CISH) are valuable tools for detecting EBV in breast cancer, although each presents certain limitations. Their combined application offers enhanced diagnostic utility and more reliable results. CISH analysis demonstrated notable variations in signal intensity and distribution patterns, including diffuse, punctate, and mixed staining. Similarly, EBV staining intensity in breast cancer tissues ranged from weak to moderate to strong, with considerable heterogeneity between samples. It appears that the role of the EBV signal intensity in breast cancer is still not clearly defined and requires further investigation.

Age represents a confirmed risk factor for breast cancer, with increased incidence in middle-aged women; however, the role of age in modulating the EBV-breast cancer relationship remains undefined. Evidence suggests that EBV positivity may correlate with more aggressive tumor characteristics, including larger tumor size, higher histological grade, hormone receptor negativity, and poorer prognosis. However, contradictory findings in the literature regarding associations between EBV and specific clinicopathological parameters warrant further investigation to establish definitive correlations.

The detection of EBV DNA in healthy control tissues could be attributed to subclinical infection, B-cell carriage, or technical factors, highlighting the importance of distinguishing

between persistent infection and active viral participation in carcinogenesis. This differentiation is critical for understanding the true role of EBV in breast cancer pathogenesis. The precise mechanisms through which EBV may contribute to breast carcinogenesis remain inadequately defined, necessitating additional research to elucidate potential viral-host interactions that could serve as therapeutic targets. Further investigation into the contradictions in current literature regarding EBV's relationship with clinicopathological characteristics would significantly advance our understanding of this virus's role in breast cancer development and progression.

5. DECLARATIONS

5.1. Study Limitations

The current study presents limitations that should be acknowledged, including a relatively modest sample size and recruitment from a single geographic region, which may restrict the generalizability of our findings to broader populations with different demographic and environmental characteristics.

5.2. Acknowledgements

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5.4. Competing Interests

The authors hereby declare that they have no competing financial, professional, or personal interests that could have influenced the design, execution, analysis, interpretation, or reporting of this research. All authors confirm that they are completely independent from any commercial entities or funding sources that may have a potential interest in the outcomes of this study.

5.6. Use of AI

The authors declare that artificial intelligence tools were utilized to assist in the translation and linguistic enhancement of the manuscript, which was originally written in Arabic. AI was employed exclusively to enhance the grammatical quality, clarity, and fluency of the English text, without altering the scientific content, presented data, or interpretations of the results. All statistical analyses, conclusions, and intellectual contributions remain the sole responsibility of the authors. The authors thoroughly reviewed the final manuscript to ensure the accuracy and integrity of all scientific content.

6. HUMAN AND ANIMAL-RELATED STUDIES

6.1. Ethical Approval

This study was conducted in accordance with the ethical standards outlined in the Declaration of Helsinki and was approved by the Human Ethics Committee of the College of Medicine, University of Misan, Iraq, under approval number MHD2025. Formalin-fixed, paraffin-embedded tissue samples were obtained from the pathology archives with appropriate anonymization to ensure patient confidentiality. Given the retrospective nature of the study and the use of archival specimens, the ethics committee waived informed consent.

6.2. Informed Consent

All individuals provided written informed consent before participating in this study. The University of Misan College of Medicine Ethics Committee approved the consent process MHD2025). The permission form clearly stated the study's goals, procedures, risks, benefits, data confidentiality, and freedom to withdraw without penalty. Participants were informed that scientific publications and conference presentations would utilize anonymized data and research conclusions. Personal identifiers were never to be shared. Before obtaining written agreement from low-literate individuals, the study methods and consent information were thoroughly conveyed. All signed consent forms are securely stored at our institution and can be requested by editorial boards under strict confidentiality guidelines.

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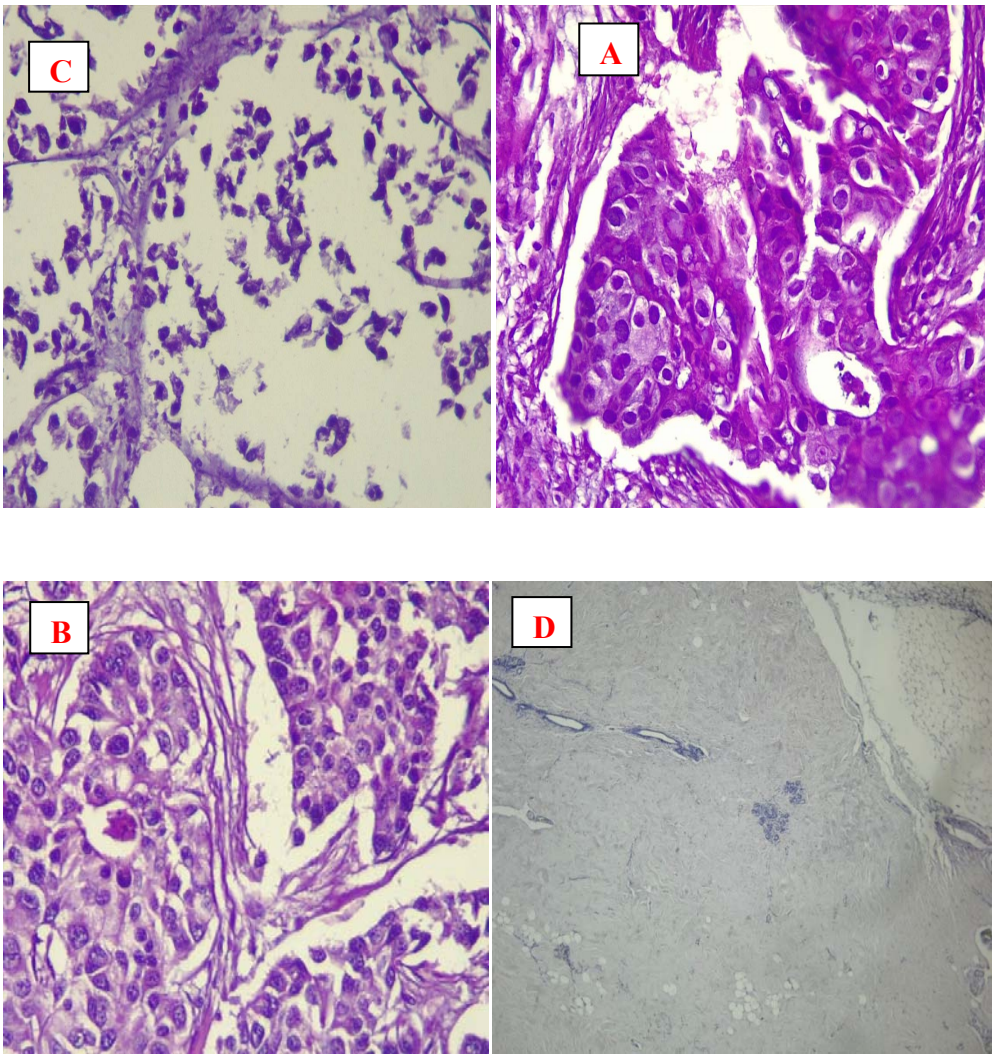

Figure (1): *Microscopic appearance of histological types of sections from tissue with invasive ductal carcinoma (IDC), stained with Hematoxylin & Eosin: A): Grade 1 invasive ductal carcinoma (X400) B) Grade 2 invasive ductal carcinoma (X400). C) Grade 3 invasive ductal carcinoma. D) Normal breast tissue.*

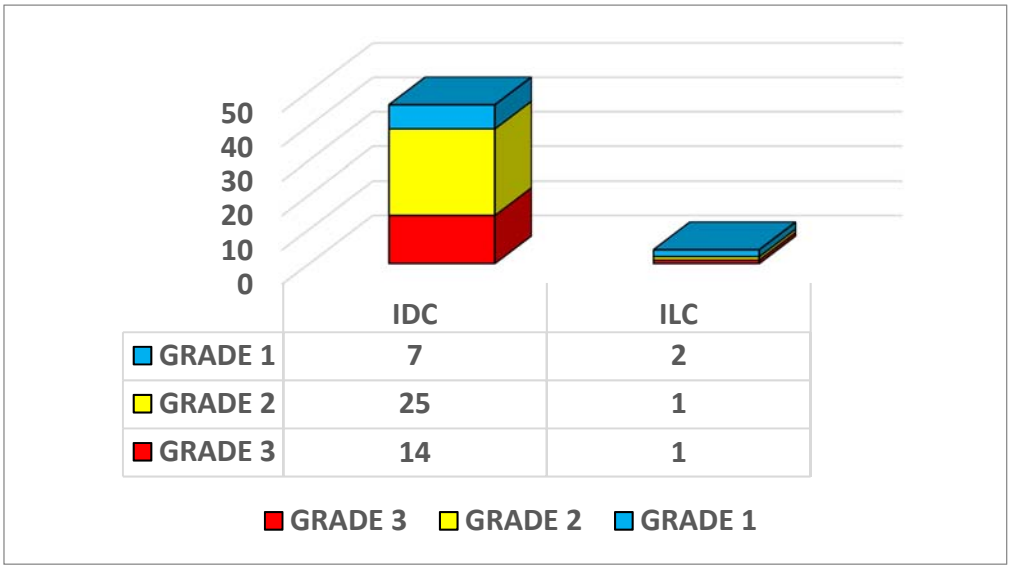

Figure 2: *Frequency Distribution of Histological Breast Carcinoma Types According to Grades*

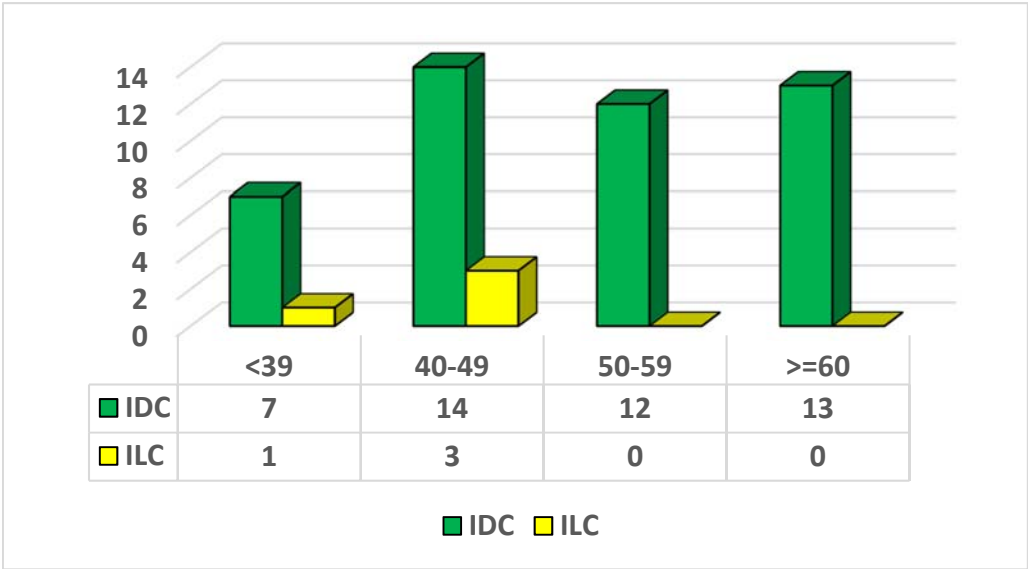

Figure (3): *Age Distribution According to Histological Types of Breast Carcinoma*

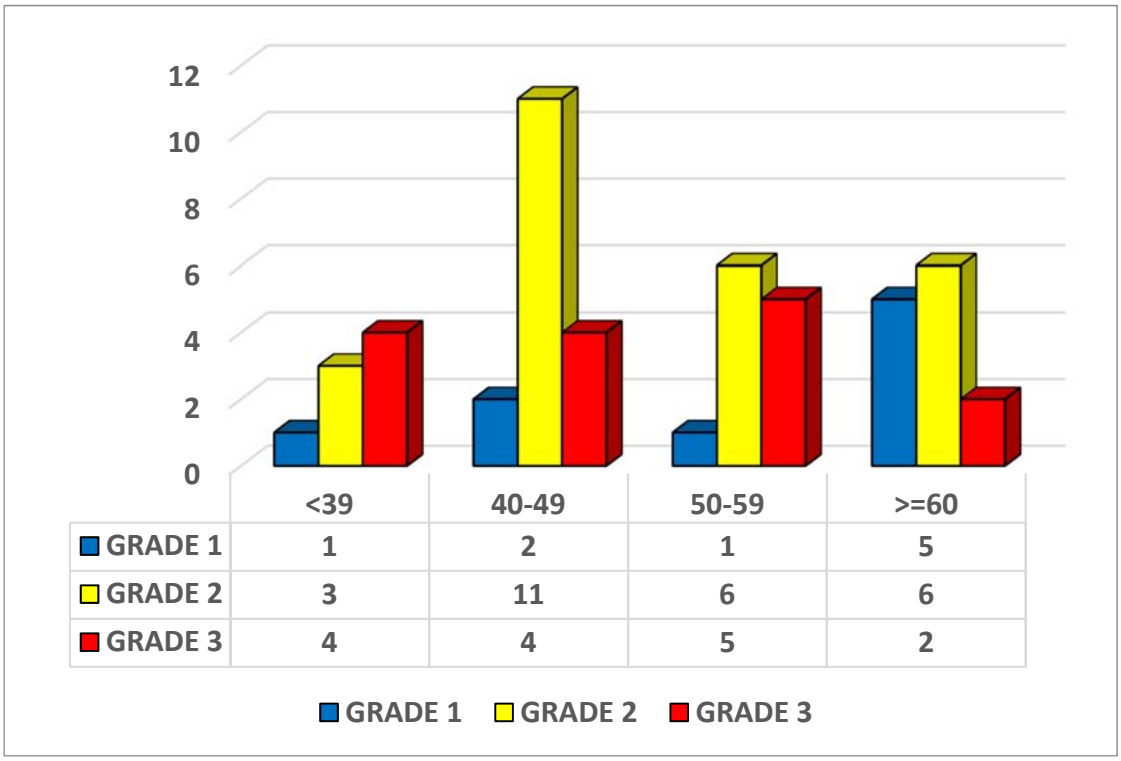

Figure (4): Age Distribution According to Histological Grades of Breast Cancer

Table 1: Total CISH and IHC results of EBV signal in the study group

Table 1: Total CISH and IHC results of EBV signal in the study group					
Study group	Total	EBV CISH results		EBV IHC results	
		+ve	-ve	+ve	-ve
Case	50	31(62%)	19(38%)	34(68%)	16(32%)
Control	30	3(10%)	27(90%)	3(10%)	27(90%)
Total	80	34	46	37	43
P-value between group	P<0.004			P<0.003	

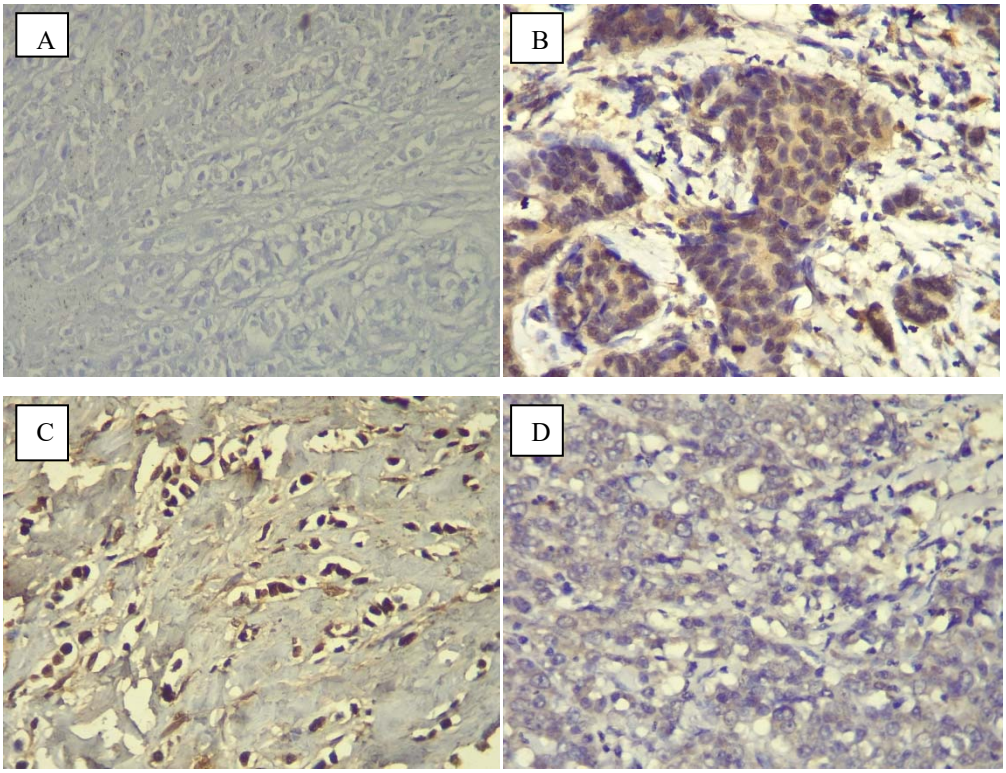

Figure (4-10): EBV EBNA-1 protein expression identified in IDC, cytoplasmic staining, A-Negative control, IHC, high power (x40). , B-Moderate intensity, IHC, high power (x40). , C-strong intensity, IHC, high power (x40). , D-Weak intensity, IHC, high power (x40).

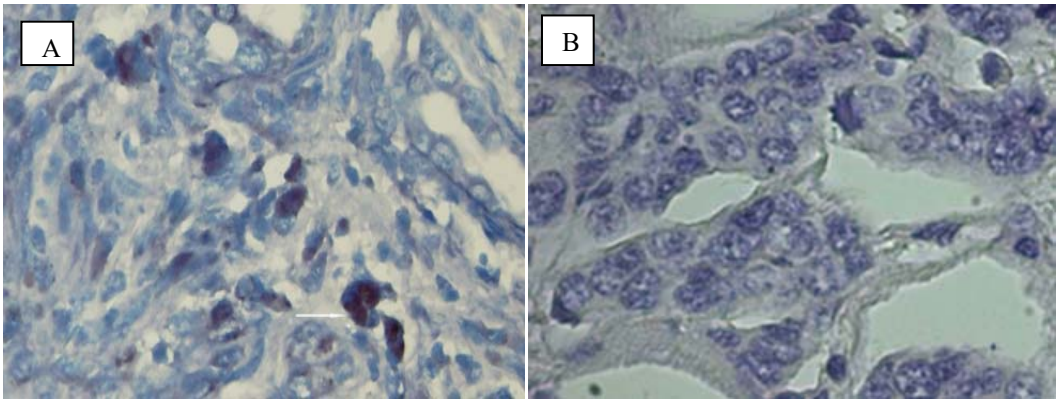

Figure (4-11): In situ hybridization staining of EBER in breast cancer section stained by DAB chromogen and counterstained with eosin (Magnification power, 400), A- EBER negative expression. (Mahjoub *et al*.,2008) B- EBER positive expression.

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