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KP1019 vs. KP1339 in Vietnam: An Economical, Chemical, and Biological Standpoint

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ABSTRACT

This literature review introduces and compares two similar ruthenium-based anti-cancer metallodrugs, KP1019 and KP133, based on the factors of costs, induction stability, cytotoxicity, and overall effectiveness. Having a higher purity and scalability through Bold Therapeutics, KP1339 has emerged as the more promising chemotherapy for commercial use. In terms of induction, while KP1339 showed higher solubility, both drugs showed little to no statistical difference in the binding of human serum albumin and transferrin. However, unique induction sites were seen within cells, with KP1019 having a slightly higher toxicity and drug uptake. Nonetheless, KP1339 is highlighted as the preferred chemotherapy due to its higher solubility, scalability, and promising clinical efficiency. Despite both drugs being in early clinical phases, KP1339's superior properties in solubility and commercial potential make it more suitable for widespread use, especially in countries with limited healthcare resources like Vietnam. In the end, continued research and developments are essential to fully unlock the therapeutic potential of ruthenium-based chemotherapies and address global cancer treatment needs.

Introduction

The Current Situation - Cancer Treatment Vs the Socioeconomic State of Vietnam.

As of 2023, Vietnam has recorded a GDP of 449.09 billion US dollars, ranking 35th globally, earning higher than many European and Middle Eastern countries like Denmark and Qatar, while also on track to becoming a developed country with a GDP growth rate of 8.09%. The GDP growth was most clearly shown within the service section with a 9.99% increase; however, the healthcare services have instead shown decreases in economic progress with a decline of 7.6% [1a]. This, along with Vietnam's surprisingly low GDP per capita of $3,300 (ranks 116th), have led to problems concerning the country's healthcare system, one of which is cancer treatment. With 165,000 novel cases and 115,000 mortalities each year (ranks 57th globally), Vietnam [1,2], along with other low-income developing countries, certainly need a cheap and effective way of battling against cancer.

Despite this ever-present need, due to a lack of resources and materials, diagnosis, and treatment of cancer is extremely difficult, especially for people living in rural areas, since the pathology services in Vietnam are heavily limited and quality services cost more than the country and people can handle [3]. Moreover, anti-cancer treatment introduced in Vietnam is scarce, with only 1 of 42 cancer drugs launched during 2011-2015 used in pharmaceutical practices within the country [4]. Other techniques for immunohistochemistry and molecular analysis are only available at certain care centers located far from rural populations. This lack of sufficient care along with Vietnam's unstable economy has resulted in unimaginable treatment costs, with therapies ranging from 40 to 50 times the average GDP per capita [3]. This is problematic because 42% of Vietnamese cancer patients admitted to having lost their life savings paying for treatment and only one in four cancer survivors was able to afford post-cancer follow-up treatment. Even for cheaper options like radiation therapy and 3D-conformal radiation therapy techniques, efficiency becomes the major drawback. For example, the cobalt-60

external radiation machines were used for most cancer cases in the country, but their efficiency and effectiveness towards cancer cells are relatively low [3]. Despite the high intensity of radiation, the machines' inaccurate targeting and constant gamma beams can cause patients to suffer various deadly side effects, such as skin burns, radiation sickness, or even cancer to other rapidly multiplying cells within the body [3a, 3b].

The Introduction of Chemotherapy and Pt/Ru-Based Anti-Cancer Drugs

Chemotherapy provides what Vietnamese patients need: an accessible yet more effective and efficient drug regime that can be delivered or administered without the need for heavy equipment compared to radiotherapy or surgery. While the cost of taking these drugs in terms of the drug and the treatment plan is more expensive compared to other forms of therapies the Vietnamese government can support 80-100% of what patients need through their government-funded health insurance [3].

By having flexibility in binding and isotopic reactivity, platinum has been used with greatest success to date to create anti-cancer treatment drugs. There are currently three Pt drugs in the clinic. Cisplatin, for example, is one of the most effective and successful metallotherapeutic drugs even today, making it the most in-demand drug that is in clinical use [5]. Soon after cisplatin's success, oxaliplatin and carboplatin were approved by the FDA and are reported to have fewer side effects than cisplatin as well as different cancer cell targets [6].

As the pharmaceutical world tries to find metals that have anti-cancer properties like that of platinum, ruthenium becomes a promising alternative. Ruthenium-based drugs like NAMI-A or RAPTA-C are currently in clinical trials and show potential against their assigned cancer(s), suggesting the possibility of future clinical use.

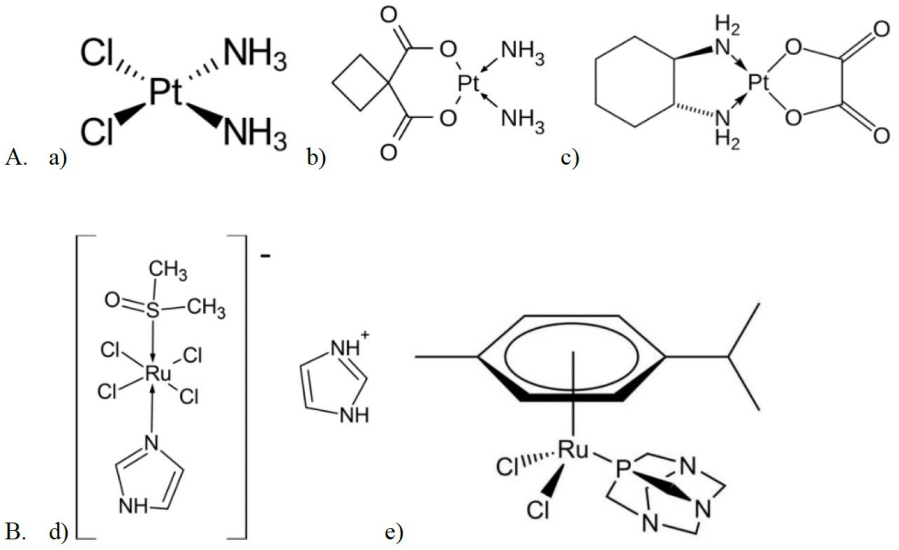

Figure 1.2. Metal-based chemotherapies
A. Platinum-based chemotherapies (from left to right): (a) Cisplatin, (b) Oxaliplatin, and (c) Carboplatin
B. Ruthenium-based chemotherapies (from left to right): (d) NAMI-A and (e) RAPTA-C

How Transition Metal Elements Show Potential for Bio-Orthogonal Drugs

Bio-orthogonal chemistry describes a non-natural chemical reaction that happens within a living body, in the case of interest here, a cancerous cell, without affecting or interfering with native biochemical reactions and processes [7]. Both platinum and ruthenium complexes have the potential to become great bio-orthogonal drugs, mostly due to the elements' chemical properties and reactivity.

Platinum has shown great medicinal success since the introduction of cisplatin. This simple coordination complex is a cis-dichlorodiammine formulation. After diffusing through the cell membrane into the cell, one or both chloride ligands are exchanged with water molecule, before the drug interacts to the N7 Position of either the Guanine or Adenine in the affected DNA allowing platinum to react with the targeted cell. This accumulation and reaction of platinum leads to cell cycle arrest, replication stoppage, and ultimately apoptosis [8].

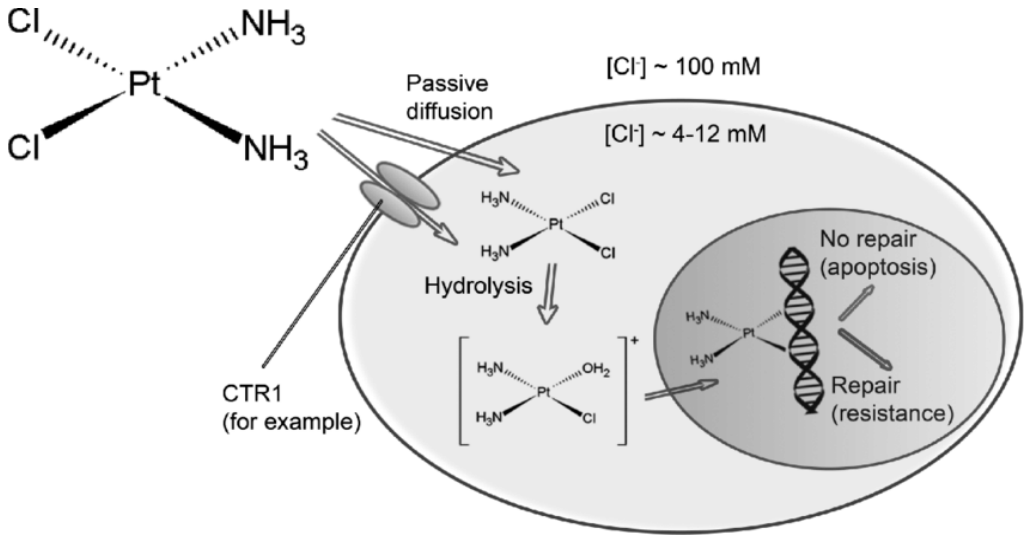

Figure 1.2.1. Cisplatin's mechanism [8]

Platinum also shows variability in the ligands that it can bond to, like the cases of oxaliplatin and carboplatin, where the derivative complexes are less cytotoxic yet also active against cancer. Finally, the reasonably short M-L bond-breaking and bond-forming time (approximately 70 minutes) has proven to be vital in the targeting of the cells, since slower ligand dissociation time means that the drug is more likely to stay intact until it can find the affected area [9].

Drugs created using ruthenium are different from their Pt counterparts since their formulations are more spatially varied than the majority of square planar platinum-based drugs. Instead, ruthenium can have different coordination numbers and subsequently different shapes, such as octahedral (CN 6) with the case of NAMI-A or tetrahedral (CN 4) like RAPTA-T, which helps tremendously with the potential to design ruthenium-based drugs for unique cancers. Another great feature of ruthenium is its variability in accessible oxidation states (III and II), which could exploit the "Activation by Reduction" effect. This theory suggests that when an inert Ru (III) compound enters a body, the cancerous environment would reduce it into Ru (II). The change in oxidation state leads to weaker electrostatic interactions between the metal and the ligands, hence making the complex more reactive and opening up the possibility of an enhanced and more concentrated delivery of the cytotoxic payload directly to the cancerous cell [10].

Comparing Non-Natural Metals to Metals That the Body Regulates

A concern when dealing with metallodrugs is how they regulate when they are induced into the body, since unnatural metals may damage organs and lead to other ultimately undesirable side effects, and this was true for some cases of modern chemotherapies.

In the case of platinum, while the metal itself already exists in the healthy human body, more specifically within the hair, kidney, and blood, as well as in the food we consume such as meats, eggs, and grains

(≈0.25 - 0.5µg/day) [11], excess platinum doses can cause serious health concerns for patients, which includes nausea, constipation, and hair loss. This entire process is non-lethal if treated properly, since excess platinum will eventually be separated through urination [11], but lowering discomfort is an aspect that is continually sought after within these drugs.

Hence, as more metals come into chemotherapeutic research, ruthenium's properties were recognized. Despite not being present in the human body, ruthenium-based drugs' mechanisms and deliveries are somewhat similar to a highly abundant and crucial metal that a human needs, iron. Both being in group 8 of the periodic table, iron and ruthenium have important similarities that help with binding and protein cell uptake, more notably the transferrin protein, with some theories claiming ruthenium can bind with other parts of transferrin and ultimately piggyback the transferrin protein alongside the iron that it binds with rather than taking iron's place [12].

However, it's also important to realize that there are differences in the degree of binding and uptake between iron and ruthenium, what these differences are remains, as yet to be determined, and is likely to vary depending on the source of ruthenium (i.e. the complex that is administered). Nevertheless, it is possible that the Fe and Ru mechanisms of biological uptake could be similar, and this may well be a help when it comes to regulation of Ru within the body and the uptake of ruthenium in the cell [12].

KP1019 and KP1339 Introduction

With ruthenium being a great alternative to the already-popular platinum, efforts are being made to take advantage of ruthenium's properties and create a chemotherapy drug that could improve patient outcomes. Until today, only four ruthenium-based compounds have found their way into clinical trials. Two of these are strikingly similar in formulation and are having some great results: KP1019 and KP1339 [13,14].

KP1019 (Figure 1.3) is a ruthenium-based anti-cancer complex discovered by Dr. Bernhard Keppler at the University of Vienna in 1989 when he was looking for a safer alternative to platinum-based drugs and experimenting with ruthenium. The negatively charged trans-[tetrachlorobis(1H-indazole) ruthenate (III)]$^-$ complex has [indazole]$^+$ as a counter ion, KP1019 has already got into phase I clinical trials and has generated promise in the treatment of colorectal cancer [15].

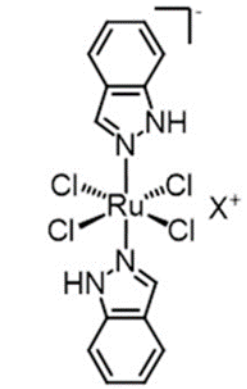

Figure 1.3. KP1019/KP1339

KP1339/NKP1339/BOLD-100 (Figure 1.3) was also developed by Dr. Keppler shortly after the discovery of KP1019 by replacing the counter ion with Na^+ creating a drug 35-fold more soluble than KP1019. The KP1339 drug has already got into phase IIA of clinical trials. This compound, however, was difficult to synthesize and the ownership as well as name were switched multiple times until Bold Therapeutics was finally able to commercially synthesize the drug and claimed it with the name BOLD-100 [16].

KP1019 and KP1339 Key Mechanisms

Both KP1019 and KP1339 are used to treat colorectal cancer via three main steps: the drug is infused into the body and binds with human protein serum transferrin in the activation site. The drugs then get activated from Ru (III) to Ru (II), increasing their cytotoxicity, inhibiting the GRP78 and promoting the activation of caspase, which leads to ER stress and ultimately the apoptosis of the cancer cells [17] (Figure 1.3.1). Compared to a well-established drug like cisplatin, the mechanism of action of the Ru drugs is quite different, in that the Ru drugs target the specific cells of binding into the transferrin protein, appear to have a special delivery system, and have a method of creating apoptosis in the cell, which creates less damage and distortion to the DNA than cisplatin [18]. Another advantage that both these ruthenium compounds have over cisplatin is the 6 available M-L bonds, or 4 coordination sites to bind with DNA. This means there are more probability that the drug can lead to caspase activation with higher reactivity and hasten the process of apoptosis quickly [8].

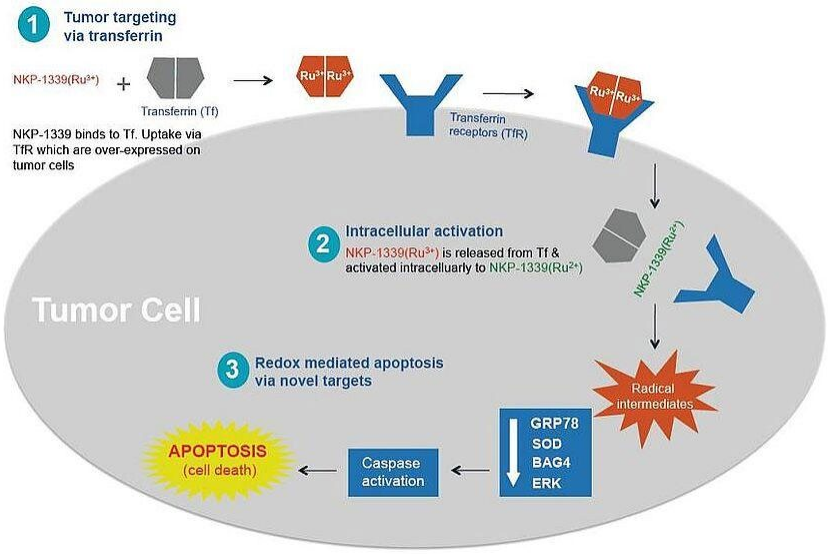

Figure 1.3.1. KP1019/KP1339 mechanism [17]

Thesis

Despite KP1019 and KP1339's similarities in formulations and mechanisms, it's unknown which drug would be presented as more suitable for Vietnam's clinical use. With a high demand in cancer treatment and a low budget, Vietnam needs a cheap but effective and less toxic drug, which will be compared and proposed within this paper.

The Comparison and Discussion

Cost

Ruthenium Costs Compared to That of Platinum

One challenge to developing anti-cancer drugs is the resources available for clinical use. Although one of the most successful drugs in the world, platinum-based drugs are too expensive due to the rarity of Pt metal, with an average cost of $900/oz, making the treatment costly [17]. Despite limited platinum resources, many other transition metals are being tested in clinical trials for cheaper and more effective metallodrugs. For example, Arsenic is the 20th most abundant metal on the Earth [18], thus Chinese and Greek healers were able to use it in cancer treatment for thousands of years at a relatively low price.

It's also worth mentioning that higher efficiency can also be a crucial factor in lowering treatment costs, as in the case of ruthenium. Ruthenium, a more abundant and consequently cheaper element than platinum, comes in with an average cost of $465 [17], although not as abundant as elements like Arsenic. Yet, when ruthenium-based drugs were put into trials, they were expected to achieve high results in efficiency, since they have an advantage over platinum: they are effective in prohibiting the spread of cancer tumours and work for a variety of cancers, thus the patients have a more likely chance of beating cancer, and a shorter treatment time, which in turn means a cheaper treatment cost [19].

With a cheaper, shorter, and more efficient way to treat cancer, low-income countries like Vietnam can afford these in clinical use, not only helping them reduce annual cancer mortalities but also motivating the country into developing those drugs, using new ligands to create more advanced drugs for different conditions and overall expanding in metallodrugs research to produce better drugs to the public.

Cost of Synthesis

Despite ruthenium being a better economic option than the predominant platinum, the price tag that comes with ruthenium is still quite high, and considering the Vietnamese low healthcare budget, a more cost-efficient drug is in substantial need.

From the process made by *Lipponer et al. (1996),* the synthesis of KP1019 requires the solution of 1g of commercially available $RuCl_3.H_2O$, 20ml of 12M HCl and 20ml of Ethanol, before heating to reflux and cooling. Ethanol is then removed and more HCl (12M HCl) is added to the solution. To this mixture, a solution of 1.87g Indazole in 30ml of 12M HCl. After being heated up to 90ºC for 15 minutes and cooling down to room temperature, a solid is formed and is filtered, before being mixed with H_2O, creating KP1019 [20]. Overall, the procedure is expected to be completed in 2-3 days and is found to be stable for at least 48 hours. Here is a table of materials used and the estimated cost for each material:

Table 1. Estimated cost for KP1019 from each ingredient used by the synthesis of Lipponer et al. (1996).

Material	Cost (per 100g of KP1019)
$RuCl_3.3H_2O$	12.2
Indazole	104.72
12M HCl	1.94
Ethanol	0.04
Diethyl Ether	0.91
TOTAL COST	119.81

The synthesis of KP1339 is concerning, however, since it is heavily dependent on KP1019. As of now, the most well-known synthesis of KP1339 is through the change of counter-ion with KP1019, with KP1019 mixing with NaCl, effectively changing the counter ion from imidazolium to sodium, creating KP1339. This should translate that the synthesis for KP1339 should cost more than that of KP1019, but recent advancements from Bold Therapeutics claim to have created a more predictable manufacturing method that stemmed from

another route of KP1339 synthesis that uses CsCl, which helps eliminate multiple steps of aqueous extraction and ultimately lowers the costs [21].

Scalability and Purity

Along with costs, KP1019 and KP1339's scalability and purity after synthesis also needs serious considerations since this can determine the drugs' commercial costs if they make it into clinical use.

In the case of KP1019, it can be seen that most of the drug's syntheses were and are still being created within labs, which can be helpful since labs in Vietnam can buy the materials locally and synthesize the drug themselves. However, with the technology currently available in Vietnam, along with the moderately long process of creating this drug, KP1019 is placed at a slight disadvantage, since the moderately long procedure of synthesizing and the shortage of technologically advanced laboratories can lead to longer drug synthesis and delivery throughout Vietnam. Purity of this drug also comes into play when it came to the processing time, since a lot of time put into this synthesis is the purification process. Even if there is a faster purification method, continuous aqueous filtration could lead to errors during the process and ultimately yield a less purified drug, despite KP1019 being quite stable [20]. With the pace of KP1019 production now and the uncertainty of labs within the country, it is likely KP1019 would be quite scarce and in turn establish a high price, both in the production and distribution, which is not beneficial for a country like Vietnam.

KP1339, on the other hand, already has a well-established manufacturer in Bold Therapeutics, and with a leading team of international partners, the drug's scalability can reach multiple countries. Furthermore, Bold Therapeutics have claimed to have developed a more predictable manufacturing process and can develop at scale, while also ensuring the quality of the drug [21]. If this is the case, the production time would be suitable for clinical use and need, since KP1339 not only has actual manufacturers that partners all around the world, which would help with the delivery and distribution, the pace and quality also ensures a high efficiency in clinical use of KP1339. With all the advantages that KP1339 has in synthesis, it can be reasonably inferred that the KP1339's traditional synthesis price should be substantially lower.

This ultimately helps when it comes to clinical use in Vietnam as well, since Bold Therapeutics have already partnered with countries like South Korea (Figure 2.1.2), which can reduce the time and cost of delivering the drugs within the South and Southeast Asian countries, many of which need new, more advanced chemotherapy [22]. However, obtaining any drug from pharma companies means that countries like Vietnam need to rely on Bold Therapeutics (or others) for production, rather than being independent. Because the materials needed for synthesis might not be readily available in Vietnam in the near future, as well as the patent rights of exclusive distribution and production of KP1339 by Bold Therapeutics, this is a problem that is hard to circumvent. Another downside to the distribution of the drug is that the scale is still not big enough for complete commercial use. Other parts of the world like Africa or the Middle East are still not partnered yet, which could affect the distribution and usage of the drug, especially since the nearest partnered site is in Europe. Even if KP1339 were to make it to the clinic, countries like Vietnam still need to wait for days if not weeks for the transfer of drugs to go through. However, in terms of scalability as of now, Bold Therapeutics have created a stable network that can be expanded later when the drug has fully gone through clinical trials.

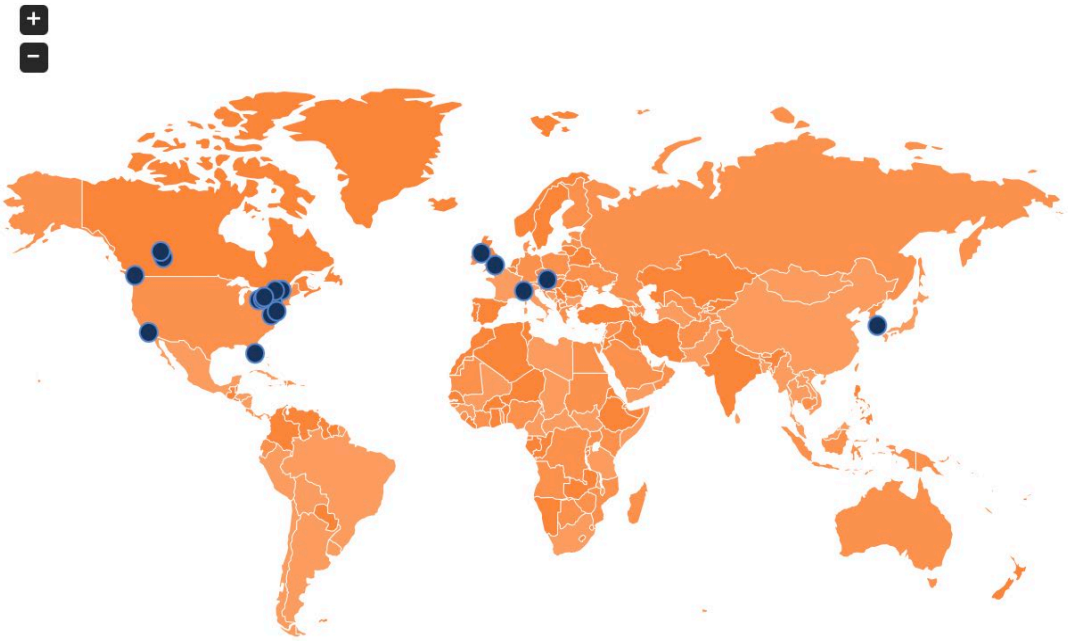

Figure 2.1.2. Bold Therapeutics partners worldwide [16]

Overall, KP1339 shows higher scale capabilities as well as more promising properties in terms of making the drug commercially available for clinical use with the help of Bold Therapeutics and their partners, whereas KP1019, although can be synthesized in labs with commercially available $RuCl_3.3H_2O$, is still inefficient in terms of producing and mass distributing, making the drug's scalability quite low. When considering the costs for Vietnam, KP1339 presents itself as a more suitable drug, since not only partners like South Korea can be the main provider and distributor of drug, helping Vietnam get better access to the drug, but also that the commercial synthesis route should decrease the price for KP1339 and give countries like Vietnam to be expose to high quality but affordable drugs.

Induction Stability

The cost and scalability of the drug is proven to be vital when tackling the distribution and maintenance of these drugs, but their effectiveness and delivery when induced, such as solubility and binding patterns, can have such important impacts that cost cannot simply cover [22].This is because even if one of the two drugs is moderately cheaper yet takes longer to reach to cancer cells or be less stable as an aqueous soluble, more dosage would then be needed to equal the effect of the other drug, hence would result in the treatment more costly than necessary.

Solubility

When looking at the formulations between KP1019 and KP1339, the main and only difference that stands out is their counter ions, and this difference can be crucial, especially when considering solubility. Solubility plays an important role in drugs, as it is not only necessary in the absorption, distribution, and excretion in the human body, but also that it means more drugs would be present within a dose and ultimately enhance the drug's effectiveness, not to mention poor-water soluble drugs can make formulation more difficult to synthesized [23].

Within KP1019, the counterion is Indazolium, an organic molecule with decent abundance in nature, which can help local laboratories easily extract and use. However, its low plasma level of only 0.3 mM can make it harder for KP1019 to be broken down when induced inside the human body [24]. This is shown when research was made to see KP1019's solubility and it was due to such low solubility that KP1019 cannot reach its optimal dosage, hence limiting the drug's effectiveness [24]. Meanwhile, KP1339's sodium counterion is more conveniently accessible, since this counter ion is abundant almost everywhere, so Na+ can be easily accessible to labs and pharmacies. It's also important to notice KP1339's solubility, since with the sodium ion, the drug can easily be broken down and thus has a higher solubility of up to 18 mM, which can be around 35-fold more than that of KP1019 [25].

Protein Binding Patterns

While solubility and plasma level are necessary when induced intravenously, the human serum proteins are major factors to the mechanism of action, more specifically, during the delivery process of both drugs, which can almost define the drugs' efficiency. A lot of studies are investigating this interaction, since proteins like transferrin or human serum albumin (HSA) are crucial in the transportation of various molecules, including metal complexes, hence they are the two primary delivery pathways KP1019 and KP1339 [26].

Studies evaluating the two proteins have shown similarities regarding the reactivity between KP1019 and KP1339. In a study conducted using inductively coupled plasma mass spectrometry (ICP-MS), the Ru-protein adducts' peak area can be seen constantly increasing over time, with transferrin having higher reactivity than HSA for both drugs, as seen in Table 2 and Figure 2.2.2 [27]. Even though this is the case, and transferrin's overexpression receptors on cancer cells can help with the selective tumor delivery, KP1019 and KP1339 are known to bind to HSA in larger degrees, suggesting the main delivery protein is within this protein [28]. An interesting discovery within the findings, however, was the stark difference in binding constant (K_D) between KP1019 and KP1339, where KP1019 is seen with a lower constant, suggesting the drug as a more binding-capable drug with HSA [29]. This, however, does not undermine KP1339's protein binding capabilities, as other components within the same study such as site marker displacement or Ultrafiltration–UV–vis spectrophotometry between the two drugs are mostly similar (shown in Table 3).

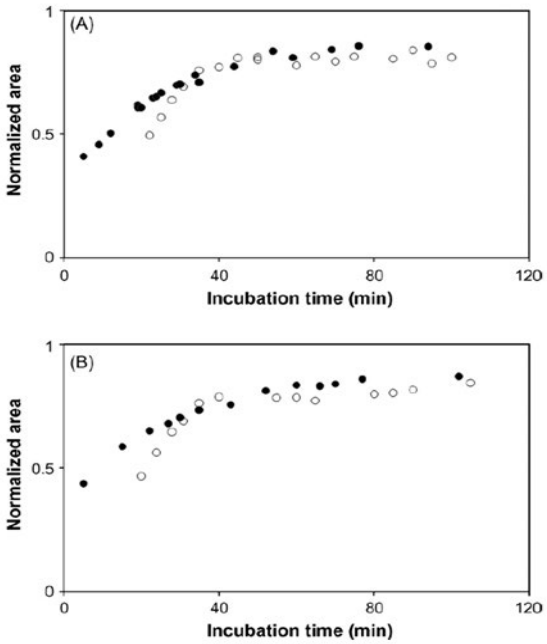

Figure 2.2.2. Interaction of (A) KP1019 and (B) KP1339 with albumin (black circles) and transferrin (open circles) expressed as the adduct peak area at variable reaction time. Drug-to-protein molar ratio: 2:1. [27]

Table 2. Reactivity of ruthenium complexes toward different proteins [27]

Complex	k ± SD (min^{-1})	
	Albumin	Transferrin
KP1019	0.0319 ± 0.0021	0.0931 ± 0.0019
KP1339	0.0316 ± 0.0018	0.0935 ± 0.0053

Table 3. Conditional binding constants (log K0) and KD of the human serum albumin (HSA)–Ru(III) complexes calculated from the spectrofluorimetric and ultrafiltration–UV–vis measurements. [29]

	KP1339	KP1019
Quenching (SD is in parentheses)		
log K′ $_{(PSEQUAD)}$	4.69 (6)	5.66 (6)
$K_{D\ (PSEQUAD)}$	20.4 μM	2.2 μM
log K′ $_{(Stern-Volmer)}$	4.66 (6); 4.92 (2)	-
$K_{D\ (Stern-Volmer)}$	21.9 μM; 12.0 μM	-
Site marker displacement		
log K′ (WF)	5.71 (8)	5.83 (10)
log K′ (DG)	5.32 (13)	5.81 (19)
log K′ (BR)	6.01 (2)	6.25 (6)
Ultrafiltration UV-vis spectrophotometry		
log K_1'	4.97 (11)	4.7 (2)
log K_2'	4.67 (5)	4.6 (2)

Intracellular Distribution

After the protein binding process is delivered to the designated tumor cell, the drug would then be distributed within the cell, which is important to the speed of treatment and ties back to the costs, which can greatly affect suitability to Vietnam.

A study conducted on the distribution patterns within the cancerous cells show significant differences between KP1019 and KP1339 [30]. As can be seen in Figure 2.2.3, the two drugs were introduced in different molarity and was let for the cell medium to absorb in different time frames, and the results showed major correlation in distribution: while KP1019 mainly spreads throughout the cytosol of the cell, KP1339 focuses more within the nuclei, and was concluded that both resulted in apoptosis, making both KP1019 and KP1339 have subsequently similar patterns and efficiency when it comes to transporting and distributing inside the cancerous cells. The only slight difference between the two drugs is the uptake within the cells, which showed KP1339 with a higher uptake than KP1019, suspecting the sodium-based drug to have more potency.

One important takeaway from this study and the drugs, however, is that this challenged the view of KP1019 and KP1339's main altering target, the DNA [31]. Unlike platinum-based anticancer metallodrugs, which focused their cytotoxicity on DNA, which can cause considerable problems in the future that might require further treatment, KP1019 and KP1339 have shown prominent signs in inhibiting that suggests safer delivery of drugs, which is greatly beneficial in the likes of countries like Vietnam.

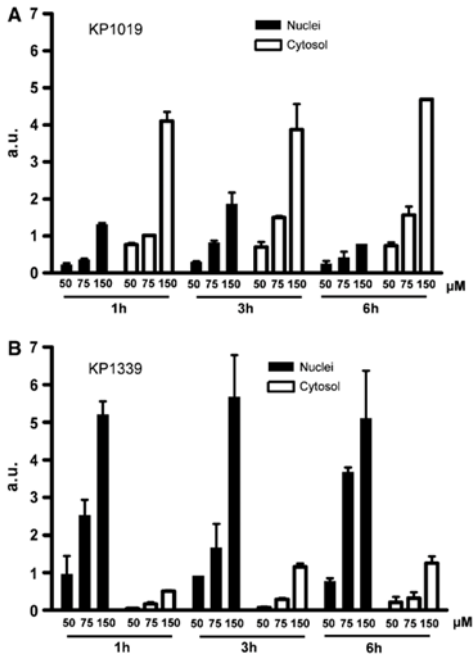

Figure 2.2.3. Drug accumulation and intracellular drug distribution. Cytosolic and nucleic ruthenium levels of a KP1019- and b KP1339- treated KB-3-1 cells were determined after 1-, 3-, and 6-h drug exposure by inductively coupled plasma mass spectrometry (ICP- MS).
[30]

This section discussed the difference in the delivery process between KP1019 and KP1339 through evaluating their solubility and plasma level, assessing their compatibility within the human body, which showed higher solubility for KP1339 than KP1019, mainly due to their counterions; determining their protein binding patterns with transferrin and human serum albumin (HSA) which resulted in similar patterns and reactivity,

although KP1019 has shown to yield higher binding constant than KP1339; and evaluating their distribution within cells, which provided insight into their preferred areas of activations and saw a higher yield in drugs for KP1339 in the nuclei, whereas KP1019 yielded less drugs throughout the cytosol, although still resulting in similar activities within the cells.

Cytotoxicity and Overall Effectiveness

The final criteria evaluated in comparing KP1019 and KP1339 is cytotoxicity, which is the last and the most vital part in the whole treatment process, not only for KP1019 and KP1339, but also for every metallodrugs in clinical use and/or in clinical trials as of now. This can be seen through the specific tumor that KP1019 and KP1339 target, colorectal cancer cells, but more limitedly, colorectal carcinoma cancer cells SW480 and HT29 that are resistant to cisplatin [32]. Being too toxic can also cause problems, as seen in the case of Vietnam's cobalt-60 radiation machines, it could lead to severe side effects and even unwanted deaths.

For the most parts, KP1019 and KP1339's cytotoxicity is within just within the perfect zone: while Ruthenium itself is highly toxic and could kill a person upon exposure, both drugs were synthesized from Ru (III) which is stable, and it's only until it reaches cancerous cells does it turns into the reactive Ru (II), through the activation by reduction theory [33]. They're also more effective in killing the cells compared to Platinum-based drugs, which was shown when KP1019 and cisplatin were used to compare the two, shown in Figure 2.3 [34, 34a].

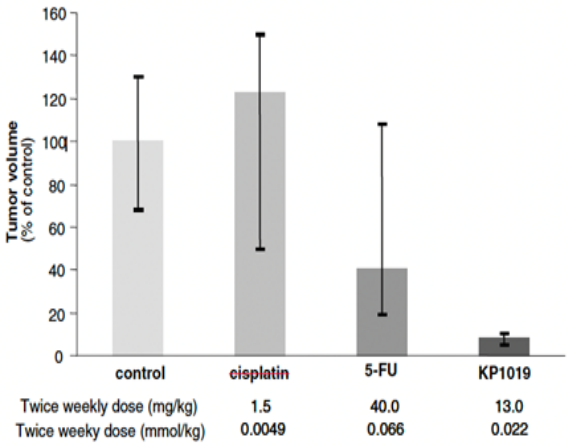

Figure 2.3. The activity of KP1019 in autochthonous colorectal tumors of the rat compared to cisplatin and 5-fluorouracil. Drugs were applied twice a week over 10 weeks. The relative tumor volume is presented in comparison to the control group (T/C% = median tumor weight of treated/median tumor weight of untreated animals · 100). Based on data from Ref. [34a],[34]

This big of a difference alone can make amazing impacts to the pharmaceutical industry, and to further improve these impacts, looking deeper between KP1019 and KP1339 in their cytotoxicity can help Vietnam access the most effective drug, helping not only Vietnam's clinical industry and potentially saving millions of lives, but this exposure to the more effective drug can also push the Vietnamese as well as the rest of the world to further study and enhance the drug, creating less toxic but highly efficient drugs for future generations. This section will investigate certain cytotoxic properties, such as amount needed to fully lead the cancerous cells to apoptosis, while also looking into potential side effects that could arise.

IC_{50} and Drug Uptake

According to the National Library of Medicine, The Half-maximal inhibitory concentration (or IC_{50}) is mostly used in the pharmaceutical industry to assess a drug's potency by measuring the amount of drug needed for a targeted process to be inhibited by half, and in this case, the concentration of KP1019 and KP1339 for a cancerous cell medium to be deduced by 50% [35], meaning if a drug's IC_{50} is low, less of the drug is needed to completely eliminate the desired cancer cells, which would result in high effectiveness rates.

A study was conducted to find the cytotoxic correlation between KP1019 and KP1339, and after being placed in numerous cancer cell medium for around 72 hours, both drugs yielded interesting results [30]:

Table 4. IC50 values after 72h [30]

	KP1019 (μM)		KP1339 (μM)	
	IC_{50}	SD	IC_{50}	SD
KB-3-1	99.3	29.3	135.3	25.8
HCC1.1	108.1	56.0	148.9	42.1
HCC1.2	82.9	0.2	88.3	26.4
Hep3B	98.4	25.3	143.1	15.3
SW480	58.2	21.4	110.2	31.7
HCT116	36.6	17.0	34.7	3.6
P31	151.2	12.9	148.0	9.2
P31/cis	147.0	8.0	150.0	0.8
VMC	56.4	9.6	77.5	6.3

As shown in Table 4, most of the values shown indicated that KP1339 was less cytotoxic compared to KP1019, with Hep3B and SW480 having the largest margin of concentration needed to inhibit the cells. This meant despite being the older drug, KP1019 is more effective in attacking the cancerous cells.

To further dive into the cytotoxic effects of both drugs, the same study conducted an experiment to consider their apoptosis patterns, which fully supported the claim made earlier that KP1019 was more cytotoxic and hence more effective dosage-wise [30].

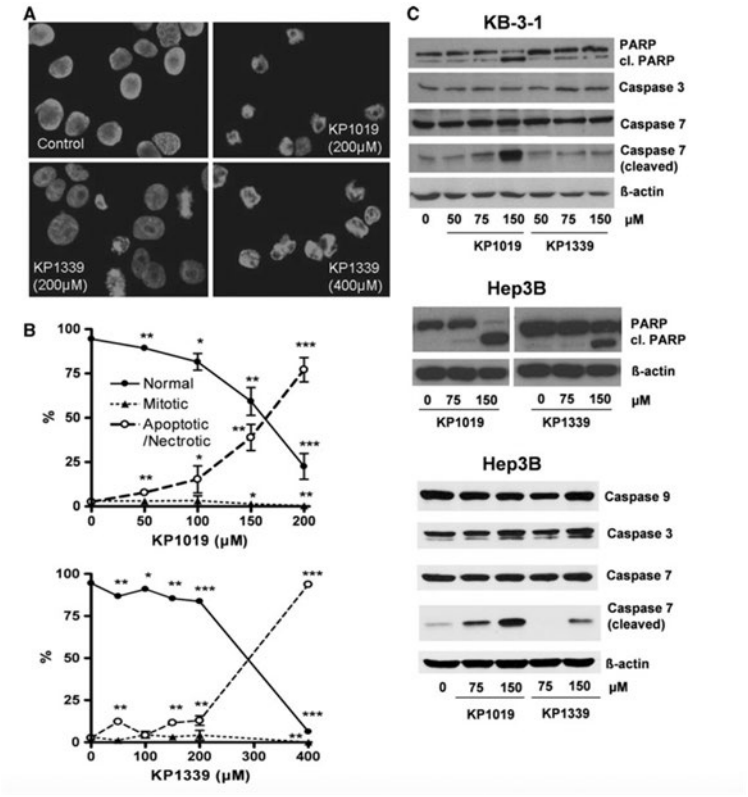

Figure 2.3.1.1. "Comparison of apoptosis-inducing potential of KP1019 vs KP1339. A. Induction of apoptosis in KB-3-1 cells was determined after treatment for 24h. 4',6-Diamidino-2-phenylindole (DAPI) staining of nuclei is shown in untreated controls and cells treated with the drug concentrations indicated. B. Morphological features of 300-500 nuclei of at least two slides for each concentration were analyzed by DAPI staining. The percentages of normal, miotic, and apoptotic nuclei at the concentrations are shown. C. Apoptosis-induced cleavage of poly(ADP-ribosyl)polymerase (PARP), caspase 7, and caspase 3 in KB-3-1 and Hep3B cells after 24h treatment" [30]-Heffeter et al

From Figure 2.3.1.1 and the study, KB-3-1 cells were induced with both KP1019 and KP1339 for 24 hours, and it was evident how KP1019 only needed a lower molarity (from 150-200 µM) to show clear signs of apoptosis induction, whereas KP1339 over double the concentration (up to 400 µM) to show evidence of apoptotic cells. After the study, the caspases of the cells were then investigated, which saw stark increase in the cleavage of caspase 7, which indicated apoptosis within cells.

KP1019 and KP1339's drug uptake in cancerous cells was also compared, which although was pronounced as not directly correlated to their cytotoxicity, can still have an impact within treatment and the drugs' potency [30,34b]. While the same study showed the cellular uptake of various cancer cell medium, which can give a broader perspective for 50 µM of each of KP1019 and KP1339, SW480 gave the most perspective, when another study looked into the uptake of these drugs within different concentrations, showing that although KP1339 showed higher uptake at lower concentrations, as it increases, KP1019 outtakes KP1339 and is induced more within the cells. This, alongside with the previous results showing KP1019 only needed a low concentration to eliminate half of the cancer cells, meant treatment only needed a small amount of KP1019 to enter the cells to activate caspase and lead to cell apoptosis. On the other hand, KP1339, although can be easily induced, needs higher concentration to create apoptotic cells.

Table 5. Total drug uptake after exposure to 50 M of KP1019 and KP1339 [30]

	KP1019		KP1339	
	Ru/10^5 cells (ng)	± SD	Ru/10^5 cells (ng)	± SD
KB-3-1	5.3	0.56	3.0	0.06
HCC1.1	7.7	2.55	5.1	0.96
HCC1.2	4.4	0.35	3.1	0.46
Hep3B	7.5	0.43	3.2	0.14
SW480	2.6	0.15	2.4	0.40
HCT116	3.0	0.3	1.4	1.5
P31	2.4	0.66	0.24	0.36
P31/cis	1.9	0.35	ND (below detection limit)	-
VMC	5.59	0.51	1.97	0.15

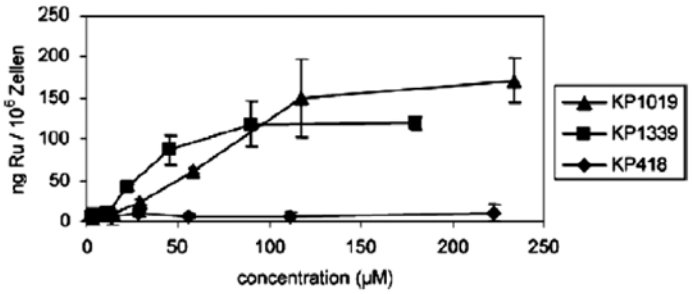

Figure 2.3.1.2. Drug uptake of KP1019, KP1339, and KP418 into SW480 carcinoma cells. [34]

Side Effects That Have Arisen

Side effects are one of the most concerning factors for patients, since chemotherapy is known to also attack other rapidly dividing cells, such as hair, bone marrow, etc. and could potentially severely damage the patients' healthy cells, leading to more treatment and would end up with higher costs, which is undesirable for pharmaceuticals not only in Vietnam, but also for other countries that are in need of better chemotherapies. Fortunately, both KP1019 and KP1339 are being tracked of side effects while they're in clinical trials.

For KP1019, the drug was used to treat 8 different tumors, and for most of the patients, the drug outputted a stable disease for up to 10 weeks, which indicated that KP1019 had prohibited the spread of these cancer cells for a moderately long time [36]. However, it is still in Phase I of clinical studies, which dealt more with animals, so further studies are still in need.

Table 6. Results in phase I trials of KP1019 [36]

Patient #	Diagnosis at study entry	Additional cycle yes/no	Dose (mg)	Outcome
1	Site: sigmoid colon Histology: adenocarcinoma	Yes (cycle 2)	25	Stable disease Duration: 9 weeks
2	Site: rectum Histology: adenocarcinoma	No	50	Not evaluable
3	Site: colon Histology: adenocarcinoma	No	50	Stable disease Duration: 10 weeks
4	Bladder cancer	No	100	Progressive disease
5	Site: liver Histology: cholangiocellular carcinoma	Yes (cycle 2)	200	Stable disease Duration: 8 weeks
6	Site: endometrium Histology: carcinoma	No	400	Stable disease Duration: 10 weeks
7	Site: left eye melanoma of the choroidea Histology: spindle B-cell melanoma	No	600	Not evaluable
8	Site: tongue Histology: carcinoma	Yes (cycle 2	600	Stable disease Duration: 8 weeks

As for KP1339, Bold Therapeutics have not yet released their results regarding the side effects of the drug within humans yet, but studies have stated that patients were experiencing disease stabilization, and only a few mild side effects [37].

In the end, KP1019 has showed higher cytotoxic elements compared to KP1339. Only needing a small amount of concentration, relatively long disease stabilization and mild side effects, KP1019 was more effective in activating apoptosis within the cells. KP1339, despite having lower cytotoxicity, still showed efficiency and was not too far behind in concentrations needed to destroy the cancerous cells.

Conclusion and the Future of KP1019 and KP1339

Both KP1019 and KP1339 have shown tremendous potential in being the next generations of anti-cancer metallodrugs: their cytotoxicity was higher than currently available chemotherapies, the reactivity can be controlled through “Activation by reduction”, and they don’t alter the cells’ DNA, resulting in less severe side effects. Having these drugs in clinical use will no doubt be a great help to world, and especially for countries that need cheap and effective drugs like Vietnam. Between the two compounds, although have shown slightly less cytotoxicity and binding strength, KP1339’s potency, pharmaceutical capabilities, and high solubility has made it more than suitable for Vietnam. Receiving KP1339 from Bold Therapeutics will not only greatly help the Vietnam’s cancer treatment and healthcare system as a whole, but it can also develop grounds for the expansion of the drug in Vietnam to other parts of the world, like within Southeast Asia, or parts where healthcare is heavily unrecognized, like parts in South America or Africa.

Realistically, however, more work and research are still needed to be placed, as both drugs have only reached Phase I-IIa. KP1019, although not as soluble or pharmaceutically compatible, is still an impactful cytotoxic anticancer agent, and if more research is conducted, KP1019 has the potential to be and influential drug in the future. This is only the start of ruthenium-based chemotherapies, and as better technology is produced and more advanced drugs are synthesised, the possibilities are endless.

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