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An Updated Review on Analytical Methods for Estimation of Ramucirumab, Decitabine and Cedazuridine, Crizotinib, Venetoclax, and Lorlatinib

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ABSTRACT

Ramucirumab, Crizotinib, Venetoclax, and Lorlatinib are effectively used anticancer drugs approved by USFDA. Ramucirumab inhibits vascular endothelial growth factor A, Crizotinib inhibits ALK phosphorylation, Venetoclax inhibits antiapoptotic proteins such as BCL-2, BCL-W and Lorlatinib is a kinase inhibitor with *in vitro* activity against ALK and several other tyrosine kinase receptors (ROS1, TYK1, FER, FPS, TRKA, FAK, FAK2, and ACK). To estimate these drugs several methods are developed. Methods include UV, HPLC, LC-MS, RP-HPLC, HPLC-MS. For Qualitative and Quantitative analysis of the anticancer drugs above mentioned analytical techniques are used for estimation. This paper provides information about analytical methods which are used for the estimation of anticancer drugs.

INTRODUCTION:

Cancer is a disease in which some of the body's cells grow uncontrollably and spread to other parts of the body. Cancer can start almost anywhere in the human body, which is made up of trillions of cells. Normally, human cells grow and multiply (through a process called cell division) to form new cells as the body needs them. When cells grow old or become damaged, they die, and new cells take their place. Tumors can be cancerous or noncancerous (benign). Cancerous tumors spread into, or invade nearby tissues and can travel to distant places in the body to form new tumors (a process called metastasis). Cancerous tumors may also be called malignant tumors. Many cancers form solid tumors, but cancers of the blood, such as leukemia's, generally do not. Benign tumors do not spread into, or invade nearby tissues. When removed, benign tumors usually don't grow back, whereas cancerous tumors sometimes do. Benign tumors can sometimes be quite large, however. Some can cause serious symptoms or be life-threatening, such as benign tumors in the brain. [1]

Ramucirumab

Ramucirumab is a human monoclonal antibody (IgG1) against vascular endothelial growth factor receptor 2 (VEGFR2), a type II trans-membrane tyrosine kinase receptor expressed on endothelial cells. By binding to VEGFR2, ramucirumab prevents binding of its ligands (VEGF-A, VEGF-C, and VEGF-D), thereby preventing VEGF-stimulated receptor phosphorylation and downstream ligand-induced proliferation, permeability, and migration of human endothelial cells.[2] It has a molecular formula of $C_{6374}H_{9864}N_{1692}O_{1996}S_{46}$ and a molecular weight of 143600.0 g/mol.[3] Ramucirumab structure was represented in Figure No. 1.

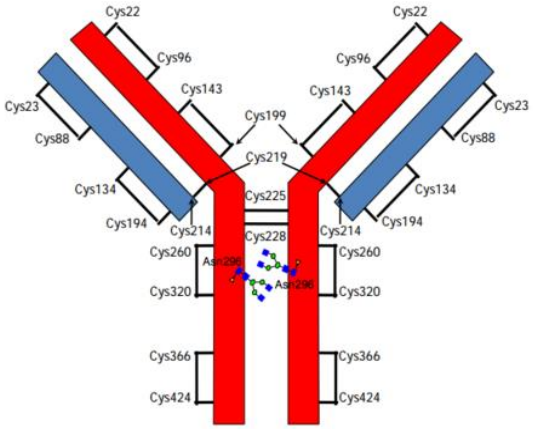

Figure No. 1: Structure of Ramucirumab

Decitabine:

Decitabine is chemically known as4-amino-1-[(2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)oxolan-2-yl]-1,3,5-triazine-2-one. Its Molecular formula is$C_8H_{12}N_4O_4$ and its molecular weight is 228.21 g/mol. The Decitabine structure was characterized in figure 2.

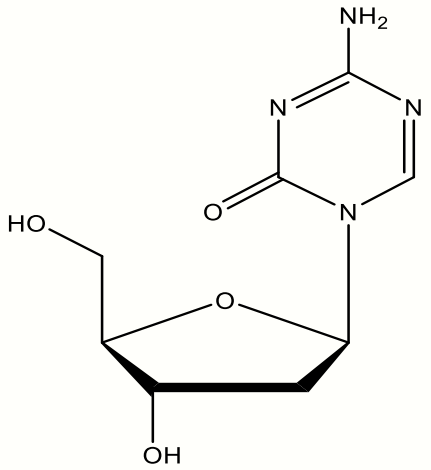

Figure No. 2: Chemical Structure of Decitabine

Cedazuridine:

Cedazuridine is chemically known as (4*R*)-1-[(2*R*,4*R*,5*R*)-3,3-difluoro-4-hydroxy-5-(hydroxymethyl) oxolan-2-yl]-4-hydroxy-1,3-diazinon-2-one. Its molecular formula is $C_9H_{14}F_2N_2O_5$ and its molecular formula is 268.21 g/mol.[4] Cedazuridine structure was denoted in figure 3.

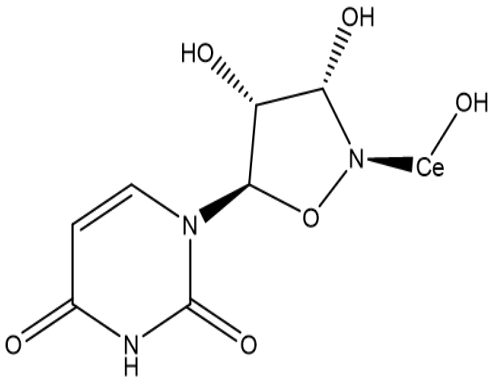

Figure No. 3: Chemical Structure of Cedazuridine

The combination of decitabine and cedazuridine is used to treat certain types of myelodysplastic syndrome (conditions in which the bone marrow produces blood cells that are misshapen and do not produce enough healthy blood cells), including chronic myelomonocytic leukemia (CMML) in adults. Decitabine is in a class of medications called hypomethylation agents. It works by helping the bone marrow produce normal blood cells and by killing abnormal cells in the bone marrow. Cedazuridine is in a class of medications called cytidine deaminase inhibitors. It helps to increase the amount of decitabine in the body so that the medication will have a greater effect.[5]

Crizotinib:

Crizotinib is chemically known as 3-[(1*R*)-1-(2,6-dichloro-3-fluorophenyl)ethoxy]-5-(1-piperidin-4-ylpyrazol-4-yl)pyridin-2-amine. Its molecular formula is $C_{12}H_{22}Cl_2FN_5O$ and molecular weight is 450.3 g/mol. [6] Crizotinib structure was epitomized in Figure 4.

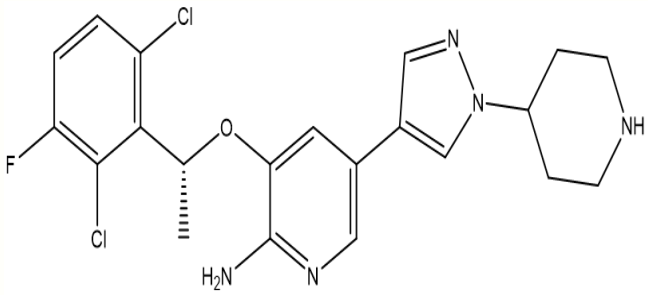

Figure No. 4: Chemical Structure of Crizotinib

Crizotinib is a tyrosine kinase receptor inhibitor. More specifically, it inhibits anaplastic lymphoma kinase (ALK), hepatocyte growth factor receptor (HGFR, c-MET), and Recepteur d'Origine Nantais (RON). Abnormalities in the ALK gene caused by mutations or translocations may lead to the expression of oncogenic fusion proteins. Patients with NSCLC, have the EML4-ALK gene. Crizotinib inhibits ALK tyrosine kinase which ultimately results in decreased proliferation of cells that carry the genetic mutation and tumor survivability. The peak serum concentration was reached in 4 to 6 hours following an oral single-dose administration. Steady-state was reached within 15 days when a dose of 250 mg twice daily was administered. The mean volume of distribution (V_{ss}) is 1772 L following intravenous administration of a 50 mg dose. Crizotinib is metabolized by CYP3A4 and CYP3A5 in which these enzymes mediate the O-dealkylation of the drug. 63% and 22% of the administered dose was recovered in feces and urine.[7]

Venetoclax

It is chemically known as 4-[4-[[2-(4-chlorophenyl)-4,4-dimethylcyclohexen-1-yl] methyl]piperazin-1-yl]-*N*-[3-nitro-4-(oxan-4-ylmethylamino)phenyl]sulfonyl-2-(1*H*-pyrrolo[2,3-b]pyridin-5-yloxy)benzamide. Its molecular formula is $C_{45}H_{50}ClN_7O_7S$ and molecular weight is 868.4 g/mol. [8] Venetoclax structure was signified in Figure 5.

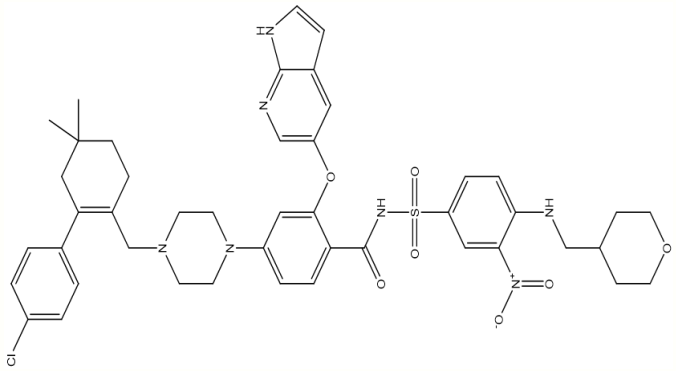

Figure No. 5: Chemical Structure of Venetoclax

Venetoclax is a protein in the B cell CLL/lymphoma 2 (BCL-2) families are the necessary regulator of the apoptotic (anti-cell programmed death) process. This family comprises proapoptotic and prosurvival proteins for various cells. Cancer cells evade apoptosis by inhibiting programmed cell death (apoptosis). The therapeutic potential of directly inhibiting prosurvival proteins was unveiled with the development of venetoclax, a selective inhibitor of both BCL-2 and BCL-2-like 1 (BCL-X(L)), which has demonstrated clinical efficacy in some BCL-2-dependent hematological cancers. Selective inhibition of BCL-2 by venetoclax, sparing BCL-xL enables therapeutic induction of apoptosis without the negative effect of thrombocytopenia. The maximum plasma concentration of venetoclax was reached 5-8 hours after the dose. The apparent volume of distribution (Vd_{ss}/F) of venetoclax ranged from 256-321 L. Venetoclax is predominantly metabolized as a substrate of CYP3A4/5. Venetoclax dose to healthy subjects, >99.9% of the dose was found in feces, and <0.1% of the dose was excreted in urine within 9 days.[9]

Lorlatinib:

Lorlatinib is chemically known as (16*R*)-19-amino-13-fluoro-4,8,16-trimethyl-9-oxo-17-oxa-4,5,8,20-tetrazatetracyclo [16.3.1.$0^{2,6}$.$0^{10,15}$]docosa-1(22),2,5,10(15),11,13,18,20-octaene-3-

carbonitrile. Its molecular formula is $C_{21}H_{19}FN_6O_2$ and molecular weight is 406.4 g/mol. [10] Lorlatinib structure was outlined in Figure No. 6.

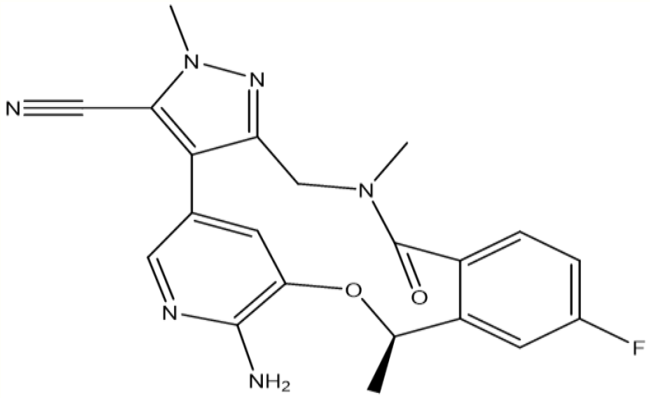

Figure No. 6: Chemical Structure Lorlatinib

Lorlatinib is a kinase inhibitor with *in vitro* activity against ALK and a number of other tyrosine kinase receptor-related targets including ROS1, TYK1, FER, FPS, TRKA, TRKB, TRKC, FAK, FAK2, and ACK. Lorlatinib demonstrated *in vitro* activity against multiple mutant forms of the ALK enzyme, including some mutations detected in tumors at the time of disease progression on crizotinib and other ALK inhibitors. Moreover, lorlatinib possesses the capability to cross the blood-brain barrier, allowing it to reach and treat progressive or worsening brain metastases as well. The overall antitumor activity of lorlatinib in *in-vivo* models appears to be dose-dependent and correlated with the inhibition of ALK phosphorylation. Lorlatinib T_{max} was 1.2 hours (0.5 to 4 hours) following a single oral 100 mg dose and 2 hours (0.5 to 23 hours) following 100 mg orally once daily at a steady state. The volume of distribution (V_{ss}) was 305 L (28%) following a single intravenous dose. Lorlatinib is metabolized primarily by CYP3A4 and UGT1A4, with a minor contribution from CYP2C8, CYP2C19, CYP3A5, and UGT1A3. Recovered in urine (<1% as unchanged) and 41% in faces (about 9% as unchanged).[11]

Table No. 1: Reported analytical methods for Ramucirumab, Cedazuridine, and Decitabine, Crizotinib, Venetoclax, and Lorlatinib

S. No.	Drugs	Method	Description	Ref. No.
1.	Ramucirumab in tablet dosage form	HPLC Method	**Stationary Phase**: YMC C18 (4.6 ×150mm), 5µm particle sized **Mobile Phase:** Methanol: Water (80:20 %v/v) **Wavelength**: 320 nm **Linearity Range:** 20 to 100 µg/ml **Flow rate:** 0.6mL/min **Injection Volume**: 20µL **Run Time:** 6min **Retention Time:** 2.497 **LOD:** 0.32µg/ml **LOQ:** 0.98µg/ml **Theoretical Plates :**4159.0	12
2.	Cedazuridine and Decitabine Combination	RP- HPLC METHOD	**Stationary Phase:** C18 (4.6×250 mm),5µm particle size **Mobile Phase:** 0.1%orthophosphoric acid buffer pH 6.5: methanol (40:60%v/v) **Wavelength:** 220nm **Flow Rate:** 1mL/min **Injection Volume:** 10µg/L **Linearity Range of Cedazuridine**: 100 to 500 µg/mL **Linearity Range of Decitabine:** 35 to 175 µg/mL **LOD of Cedazuridine:**2.69µg/mL **LOQ of Cedazuridine:** 8.15µg/mL **LOD of Decitabine:** 1.55µg/mL **LOQ of Decitabine:** 4.68µg/mL	13

3.	Crizotinib	LC-MS /MS	**Stationary Phase:** Kinetex C18 column at 40°C 50mm×2.1mm, 2.6 µm **Mobile phase**: Methanol (Solvent A) and 0.3% Formic acid in Water (Solvent B) **Internal Standard:** Apatinib **Method:** Electrospray Ionization Mode **Flow Rate**: 0.3mL/min **Linearity Range:** 20- 8000 ng/mL **The volume of Injection:** 1µg/L **LLOQ:** 20 ng/mL **Method:** Electrospray Ionization Mode **Transitions:** m/z 450.1m/z→260.1 for crizotinib and m/z 398.2 m/z 212.0 **Plasma:** Mice Plasma **Extraction Method:** Protein Precipitation Method	14
4.	Crizotinib	HPLC –UV Detector	**Stationary Phase**: YMC ODS C18 250 mm×4.6mm, 5 µm **Mobile phase:** Methanol: Water containing 0.1% orthophosphoric acid (50:50%v/v) **Wavelength**: 267 nm **Linearity Range:** 20.41- 2041.14 ng/mL **Flow rate:** 0.6mL/min **Injection Volume**: 20µL **Run Time:** 10 min **Retention Time:** 6.86 **LLOQ:** 20 ng/mL **Plasma:** Human Plasma	15

			Extraction Method: Liquid- Liquid Extraction Method	
5.	Venetoclax	HPLC-Tandem Mass Spectrometry	**Stationary Phase**: Zorbax SB-C18, 75 mm×4.6mm, 3.5 µm. **Mobile Phase**: Isocratic, Methanol: 5mM Ammonium acetate (70:30v/v) **Flow Rate:** 0.6mL/min **Linearity Range:** 10 to 10000pg/mL **Transitions:** m/z 868.12 ⟶ m/z 321.54 and 876.9 ⟶ 329.7 **Internal Standard:** Venetoclax –D8 **Method:** Electrospary ionization Method **Plasma:** Human Plasma **Extraction Method:** Liquid Liquid Extraction Method	16
6.	Venetoclax	UHPLC by AQbD Method	**Stationary Phase**: BEH C18 150mm×2.1mm ,1.7 µm **Mobile phase:** Ammonium bicarbonate (A) and 95% Acetonitrile (B) **Linearity Range:** 8 to 12 µg/mL **Flow rate:** 00.35 – 0.45 mL/min **Run Time:** 9 min **Retention Time:** 5.005 **LOD:** 0.075 µg/mL **LOQ:** 0.188 µg/mL	17
7.	Lorlatinib	LC-MS	**Stationary Phase**: C18 column by gradient elution **Mobile Phase:** 0.1% Formic acid solution (A) and methanol (B) **Internal standard:** Afatinib –d6 **Method:** Electron Spray Ionization **Flow Rate:** 0.5mL/min	18

			Transitions: m/z 407.28[M+H] For lorlatinib and m/z 492.10 $[M+H]^+$for IS. **Plasma:** Serum	

CONCLUSION:

This review article presents with Physico-chemical properties and Pharmacological actions of anticancer drugs recently approved by USFDA. The presented review depicts the information about the various methods available in the literature for the determination of Ramucirumab, Cedazuridine, and Decitabine, Crizotinib, Venetoclax, and Lorlatinib. According to this review, it was concluded that the different analytical methods are reported for estimation of selected drugs HPLC, HPLC-MS UPLC, and LC-MS. Hence all these methods are used to estimate and validate the anticancer drugs simply, economic methods, precise, reproducible, and accurate methods. This review will help in the future to develop the analytical methods for the selected drugs and also gives knowledge about the characteristics of both drugs.

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