Comparing the Safety of Proteolysis Targeting Chimeras (PROTACs) and Small Molecule Inhibitors (SMIs) in Treating Cancer

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Introduction

Oncology is an ever-changing field of medicine with innovative treatments being constantly developed. A novel treatment that has seen major success is Proteolysis Targeting Chimeras (PROTACs), developed by Sakamoto et al. in 2001 [1]. Compared to other cancer treatments, PROTACs have shown to be more efficient in suppressing tumour growth [2]. However, given the recency of this technology, its safety concerns have not been extensively evaluated. In this paper, the currently known safety concerns of PROTACs and small molecule inhibitors (SMIs), a currently used cancer treatment, will be reviewed to examine if PROTACs could truly transform the field of oncology.

Proteolysis Targeting Chimeras (PROTACs)

PROTACs are heterobifunctional molecules that suppress tumour growth by accessing the body's ubiquitin-proteasome system [2]. Using their unique mechanism of action, PROTACs connect a protein of interest (POI) ligand to an E3 ligase via a linker, which ultimately leads to the proteasome initiating protein degradation (Figure 1) [2, 3]. The first advantage of PROTACs is that they are extremely efficient as they can precisely target any protein and avoid resistance development due to gene mutations in a POI [2]. Its enhanced selectivity has been demonstrated through a comparison between the selectivity profile of Bruton's tyrosine kinase (BTK)-inhibiting drug, Ibrutinib, and a BTK-targeting PROTAC. BTK plays a key role in the development of B-cell lymphoma (BCL), a type of cancer in which the body makes an abnormal amount of B cells (Figure 2) [4]. The results indicated that the PROTAC, contrary to the inhibitor, exclusively targeted BTK [5]. Additionally, PROTACs have greater anti-proliferative efficacy and potency compared to SMIs as demonstrated by a study on human epidermal growth factor receptor 2 driven breast cancer, targeted using both PROTACs and SMIs [6]. Lastly, PROTACs can be given in low concentrations due to their catalytic function (Figure 1A) [7].

Safety Concerns of PROTACs

Despite PROTACs' long list of advantages, they are not entirely immune to safety risks. Several studies have observed off-target protein degradation—a phenomenon where a PROTAC ligand is not specific to the target protein—in PROTACs [7, 8]. This can potentially damage healthy tissues and can lead to cardiovascular, hepatic, and neuronal toxicities [3]. However, the risk of off-target protein degradation is strongly dependent on the type of POI ligand. For example, off-target binding activity was observed in PROTACs derived from immunomodulatory drugs, whereas BTK-targeting PROTACs, as mentioned previously, were able to exclusively degrade the POI [3, 5].

Off-target protein degradation can potentially be prevented using the multiplexed isobaric tandem mass tag labelling approach. This technique simultaneously identifies and counts thousands of proteins, allowing oncologists to precisely monitor degradation activity in patients [3]. According to a study, this approach was able to identify proteins with a significantly low false positive rate (<1%) thus proving to be effective and reliable [9]. Nevertheless, additional research is crucial for the advancement of PROTACs in order to understand how off-target protein degradation will affect the patient [3].

Another safety concern of PROTACs is called the hook effect, the phenomenon in which binary complexes (POI-PROTAC or E3 ligase-PROTAC) form instead of the optimum ternary complex (POI-PROTAC-E3 ligase) (Figure 1B) [7]. The hook effect is caused when high concentrations of PROTACs are given to the patient, which leads to a decrease in PROTAC efficiency and an increase in off-target protein degradation activity [3]. According to a study by Roy et al., high-affinity ligands of the POI increase the amount of fully formed or fully unformed complexes instead of partially bound complexes, thus reducing the risk of the hook effect [10]. This also counteracts the effect where high concentrations of PROTACs reduce the amount of fully formed complexes [10].

Small Molecule Inhibitors (SMIs)

SMIs are small drugs (<1000 Da) that can easily enter cells and restrain the activities of specific intracellular molecules thereby preventing the proliferation of cancer cells [11, 12].

There are several classes of SMIs. Kinase inhibitors, commonly used to treat lung cancer, block the signal transduction pathways of protein kinases which play a key part in the division, growth, survival, and migration of cancer cells (Figure 3) [13]. Other classes of SMIs include BCL-2 inhibitors, epigenetic inhibitors and more, which act in a similar manner to kinase inhibitors [14]. Contrary to PROTACs, which are currently administered intraperitoneally, subcutaneously, or intravenously, SMIs can be administered orally to cancer patients due to their smaller size [15, 16]. Oral administration is a significant advantage as it allows a controllable and minimally invasive drug delivery as well as increased patient compliance [17].

Safety Concerns of SMIs

There are two main concerns of SMIs: drug resistance and limited target proteins. At the start of the treatment, drug-sensitive cancer cells dominate, allowing the inhibitor to efficiently suppress proliferation [14]. However, in the later stages of treatment, cancer cells with mutations dominate and develop resistance [14]. This is demonstrated in a study of patients with non-small cell lung cancer (NSCLC) in which less than 20% of patients were sensitive to the SMI due to the development of drug resistance [18]. Resistance can cause damage to patient health and increases the risk of mortality [14]. A potential solution to this is rational sequential therapy—a method in which different generations of inhibitors are used to overcome drug-resistant mutations [14]. This therapy has shown to be partially effective for anaplastic lymphoma kinase (ALK) inhibitors. Three generations of ALK inhibitors were administered to patients with NSCLC and resulted in an increased survival rate [14]. However, this method may not be promising as the third generation of ALK inhibitors were ineffective in patients with the ALK L1198F mutation [14].

Another disadvantage of SMIs is that they target a limited range of proteins. For SMIs to successfully target a protein, the protein must have binding pockets or active sites; however, approximately 75% of the human proteins do not have active sites [2, 15]. Unlike SMIs, PROTACs can target any protein by simply altering the length and structure of the linker [14]. Moreover, SMIs are required in significantly higher concentrations than PROTACs to maintain efficacy [15]. Higher doses of the drug can harm the patient's well-being and cause numerous

side effects [15]. Additionally, it can overwhelm the patient in terms of dosing schedule—limiting one's quality of life [15].

Comparison of PROTACs and SMIs

Based on current literature, PROTACs are a more effective treatment option as the advantages of PROTACs are incomparable to those of SMIs. Multiple studies have shown that PROTACs are more efficient than SMIs in preventing the division, growth, survival, and migration of cancer cells, due to their ability to completely degrade proteins [2, 5, 6]. Moreover, PROTACs have higher specificity and can be administered in low doses, making them considerably less harmful to the patient [7]. Despite SMIs' key advantage of oral administration, the advantages of PROTACs outweigh those of SMIs [15].

Furthermore, the safety concerns of PROTACs are far less detrimental than those of SMIs. The safety concerns of SMIs—drug resistance and limited target proteins—result in lower efficiency, which can increase the risk of cancer cells spreading more rapidly [14, 15]. Additionally, the safety concerns of PROTACs are more preventable than those of SMIs. While the preventative measures for SMIs' safety concerns were not effective in all patients, those for PROTACs showed promising results [8, 14]. It can be expected, however, that the preventative measures for all safety concerns of PROTACs and SMIs may make significant advancements in the near future, as they are still being actively researched and developed.

Future of PROTACs and SMIs

Both PROTACs and SMIs are in the midst of several clinical trials. As of 2021, many companies have moved their PROTAC drugs (total of 13 drugs) into Phase 1 clinical trials and several have shown promising results so far [3, 19]. PROTACs have made significant progress and are expected to expand over the field of oncology in the near future [1]. Over the last two decades, SMIs have advanced greatly as well. SMIs were first approved in 2001 for clinical use, and as of 2020, the number has increased to 43 approved SMI drugs [12]. Currently, SMIs are on the lead for clinical trials with multiple drugs in Phase 2 and 3 trials [12]. With these inhibitors

moving towards pharmaceutical advancement, scientists hope to broaden the range of proteins that SMIs can target.

Conclusion

The field of oncology has seen outstanding growth over the past few decades with the development of two cancer treatments: PROTACs and SMIs. Despite entering the field of oncology for a short period of time, both treatments have proven to be efficient in improving patients' quality of life to an extent that appears to be incomparable to existing treatments. However, neither PROTACs nor SMIs are without various safety risks. Current research has led to the conclusion that PROTACs are potentially safer than SMIs due to their numerous advantages that overrule the relatively few safety concerns. Analyzing PROTACs' current success as well as their anticipated future, it can be expected that PROTACs may truly revolutionize the field of oncology.

References

1. Sakamoto KM, Kim KB, Kumagai A, Mercurio F, Crews CM, Deshaies RJ. Protacs: Chimeric molecules that target proteins to The Skp1-Cullin-F box complex For ubiquitination and degradation. Proc Natl Acad Sci. 2001Jul17;98(15):8554–9.

2. Khan S, He Y, Zhang X, Yuan Y, Pu S, Kong Q, et al. PROteolysis TArgeting Chimeras (PROTACs) as emerging anticancer therapeutics. Oncogene. 2020May31;39(26):4909–24.

3. Moreau K, Coen M, Zhang AX, Pachl F, Castaldi MP, Dahl G, et al. Proteolysis‐targeting chimeras in drug development: A safety perspective. Br J Pharmacol. 2020Feb5;177(8):1709–18.

4. Qi S, Dong J, Xu Z, Cheng X, Zhang W, Qin J. PROTAC: An Effective Targeted Protein Degradation Strategy for Cancer Therapy. Front Pharmacol. 2021May7;12.

5. Sun X, Gao H, Yang Y, He M, Wu Y, Song Y, et al. PROTACs: great opportunities for academia and industry. Sig Transduct Target Ther. 2019Dec24;4(1).

6. Burslem GM, Smith BE, Lai AC, Jaime-Figueroa S, McQuaid DC, Bondeson DP, et al. The Advantages of Targeted Protein Degradation Over Inhibition: An RTK Case Study. Cell Chem Biol. 2018Jan18;25(1):67–77.

7. Edmondson SD, Yang B, Fallan C. Proteolysis targeting chimeras (PROTACs) in 'beyond rule-of-five' chemical space: Recent progress and future challenges. Bioorg Med Chem Lett. 2019Jul1;29(13):1555–64.

8. Bondeson DP, Smith BE, Burslem GM, Buhimschi AD, Hines J, Jaime-Figueroa S, et al. Lessons in PROTAC Design from Selective Degradation with a Promiscuous Warhead. Cell Chem Biol. 2018Jan18;25(1):78–87.

9. McAlister GC, Nusinow DP, Jedrychowski MP, Wühr M, Huttlin EL, Erickson BK, et al. MultiNotch MS3 Enables Accurate, Sensitive, and Multiplexed Detection of Differential Expression across Cancer Cell Line Proteomes. Anal Chem. 2014Jun3;86(14):7150–8.

10. Roy RD, Rosenmund C, Stefan MI. Cooperative binding mitigates the high-dose hook effect. BMC Syst Biol. 2017Aug14;11(1).

11. Khera N, Rajput S. Therapeutic Potential of Small Molecule Inhibitors. J Cell Biochem. 2016Nov4;118(5):959–61.

12. Bedard PL, Hyman DM, Davids MS, Siu LL. Small molecules, big impact: 20 years of targeted therapy in oncology. The Lancet. 2020Mar28;395(10229):1078–88.

13. Coleman WB, Tsongalis GJ, Chen X, Jin J. Small Molecule Inhibitors. In: The Molecular Basis of Human Cancer. 2nd ed. New York, NY: Humana Press; 2017. p. 771–95.

14. Zhong L, Li Y, Xiong L, Wang W, Wu M, Yuan T, et al. Small molecules in targeted cancer therapy: advances, challenges, and future perspectives. Sig Transduct Target Ther. 2021May31;6(1).

15. An S, Fu L. Small-molecule PROTACs: An emerging and promising approach for the development of targeted therapy drugs. EBioMedicine. 2018Sep14;36:553–62.

16. Smith WM, Purvis IJ, Bomstad CN, Labak CM, Velpula KK, Tsung AJ, et al. Therapeutic targeting of immune checkpoints with small molecule inhibitors. Am J Transl Res. 2019Feb15;11(2):529–41.

17. Homayun B, Lin X, Choi H. Challenges and Recent Progress in Oral Drug Delivery Systems for Biopharmaceuticals. Pharmaceutics. 2019Mar19;11(3):129.

18. Metro G, Finocchiaro G, Toschi L, Bartolini S, Magrini E, Cancellieri A, et al. Epidermal Growth Factor Receptor (EGFR) Targeted Therapies in Non- Small Cell Lung Cancer (NSCLC). Rev Recent Clin Trials. 2006;1(1):1–13.

19. Mullard A. Targeted protein degraders crowd into theclinic. Nature Reviews DrugDiscovery. 2021Mar18;20(4):247–50.

Appendix

Figure 1A

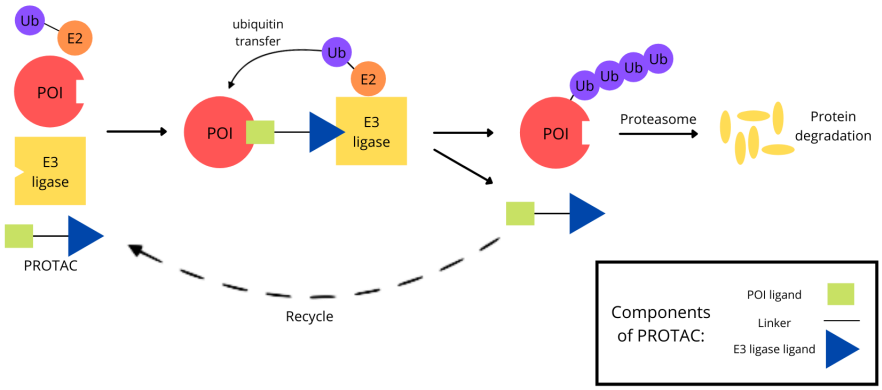

Figure 1B

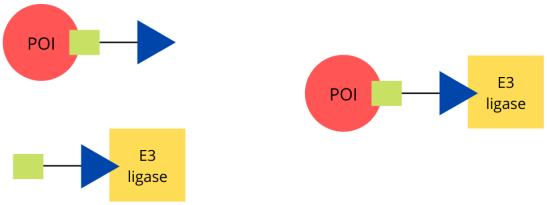

Figure 1A, 1B. PROTAC. (A) The mechanism of PROTAC and the process of ubiquitination. (B) Binary complexes (left) and optimum ternary complex (right). Figure adapted from Sun et al., 2019.

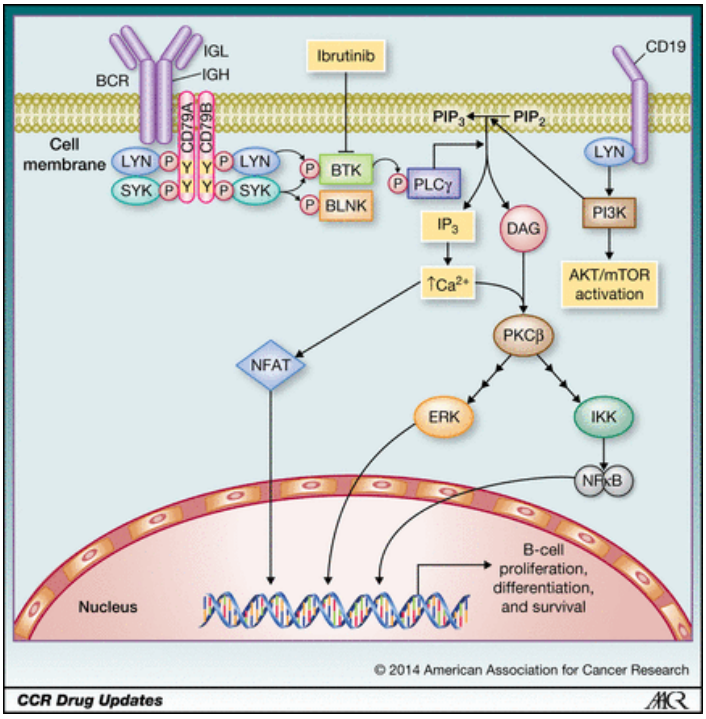

Figure 2. A mechanistic diagram of Ibrutinib, a BTK inhibitor. Figure obtained from Herrera AF, Jacobsen ED, 2014.

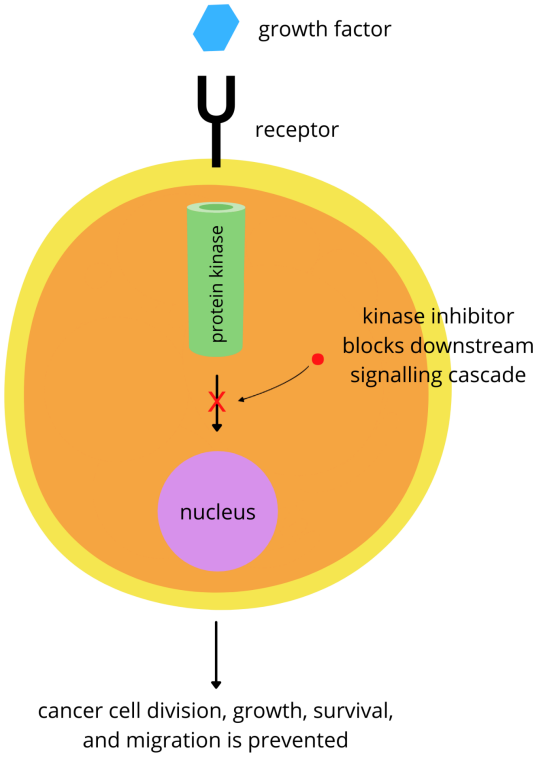

Figure 3. The mechanism of kinase inhibitors [13].

Appendix References

1. Sun X, Gao H, Yang Y, He M, Wu Y, Song Y, et al. PROTACs: great opportunities for academia and industry. Sig Transduct Target Ther. 2019Dec24;4(1). Figure 1, Mode of action of PROTACs.

2. Herrera AF, Jacobsen ED. Ibrutinib for the Treatment of Mantle Cell Lymphoma. Clin Cancer Res. 2014Oct30;20(21):5365-71. Figure 1, Ibrutinib inhibits BTK in the BCR pathway.

3. Coleman WB, Tsongalis GJ, Chen X, Jin J. Small Molecule Inhibitors. In: The Molecular Basis of Human Cancer. 2nd ed. New York, NY: Humana Press; 2017. p. 771–95.