Biorthogonal combined gene editing technology being applied to tumor treatment

Hanyue Wei

School of Life Science and Technology, China Pharmaceutical University, Jiangsu, 211198, China

Abstract. Because of the special complexity of tumor treatment, it has been a hot topic in medical research for a long time. Although CRISPR system assisted immunotherapy has been shown to be a good option, it still lacks the defect of targeting. The discovery of biological orthogonal reaction provides a new method for attenuating and enhancing the efficacy of tumor immunotherapy. In this paper, biological orthogonal reaction is introduced, and the application of biological orthogonal technology and gene editing technology in tumor therapy is studied. The study confirmed that biological orthogonal response can be used as an assistant to enhance the efficacy of gene editing technology mediated tumor targeted therapy and reduce the side effects, which provides a new idea and method for tumor therapy. Because of its high stability as well as extremely fast reaction rate, the newly discovered pentavariant diheterocyclic monosubstituted tetrazine can be considered in combination with gene editing technology for protein anchoring, controllable activation of T cell epitopes, construction of modular platforms or participation in the regulation of base editors in the future to achieve the purpose of tumor treatment.

Keywords: tumor treatment, biological orthogonal technology, gene editing technology, protein anchoring, T cell epitopes, modular platforms.

1. Introduction

Due to its special complexity, cancer treatment has long been an enduring first-line research hotspot in biomedical research. At present, CRISPR system-assisted immunotherapy, i.e., genome editing of CRISPR-associated protein (CRISPR/Cas) systems, has proven to be an attractive option for cancer treatment. However, the lack of tumor targeting of the CRISPR system leads to the "on-target but off-tumor" effect, which not only limits the efficacy of anti-tumor immunotherapy, but also induces serious toxic side effects, resulting in lower-than-expected efficacy. However, biorthogonal reaction can improve the delivery accuracy of therapeutic drugs and avoid off-target effects in tumor targeted therapy.

Bioorthogonal reactions (sometimes referred to as live-cell chemical modifications) describe a group of chemical reactions that can occur within a biological system without obstructing the body's normal biochemical functions. which is usually fast, efficient, highly selective, and has no side effects on endogenous functional groups. The discovery of biorthogonal reactions has opened a bridge between chemistry and biology, allowing scientists to manipulate the fate of molecules in the microscopic world of cells and tissues with unprecedented precision. The categories of biorthogonal reactions include native chemical ligation, Staudinger linkage, tetrazine linkage, metal-catalyzed coupling reactions, strain-facilitated [3+2] reactions, copper-catalyzed azide-alkyne cycloaddition, oxime and hydrazone linkage, and light-induced biorthogonal coupling and shearing reactions, and the discovery and further research of these reactions have shown broad application prospects in many fields such as life science research, pharmaceutical research and development, and clinical diagnosis and treatment. In this article, we will introduce biorthogonal reactions and try to combine biorthogonal technology with gene editing to apply it to cancer treatment. The purpose of this research is to confirm if biorthogonal and gene editing technology-mediated tumor immunotherapy is feasible, and to provide a new idea for improving the efficacy of tumor targeted therapy and reducing toxicity and side effects.

2. Introduction to Bioorthogonal Click (BOC) Chemistry

2.1. Research history and progress

The research boom of biorthogonal reaction began in 2000 when Bertozzi's group developed the azide-phosphine ester reaction (i.e., the Staudinger coupling reaction) based on the Staudinger reduction reaction and use it to alter the cell surface chemically (Bertozzi, 2000). Since then, over a dozen biorthogonal processes for living cells have been identified, such as the Staudinger junction reaction, the ring tension-induced azide-alkyne cycloaddition reaction, and the ligand-copper complex-catalyzed azide-alkyne cycloaddition reaction. These initial biorthogonal reactions are single conjugated reactions, which are mainly used in live-cell imaging, biomics analysis, disease diagnosis, drug development, and another research. With further research, in 2013, Robillard's group found that tetraazine molecules can trigger the removal reaction of trans-cyclooctene groups under physiological conditions to release amino groups (Robillard,2013). Since then, a new concept of biorthogonal shearing reaction has been discovered and proposed, which has shown strong advantages and prospects in the design of prodrugs, controlled-release antibody-drug conjugates, and the functional regulation of biological macromolecules such as proteins, sugars, and nucleic acids. Finally, the further development and application of biorthogonal IEDDA reaction are discussed and prospected. The principle of IEDDA reaction click release is shown in the figure 1.

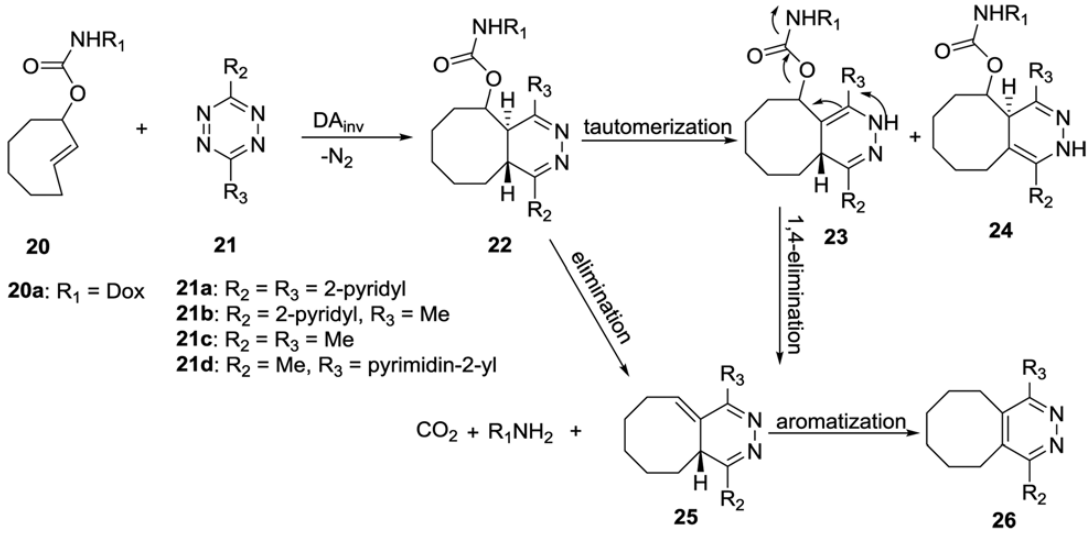

Figure 1. Trans-cyclooctene (TCO) and tetrazine undergo an inverse-electron-demand Diels-Alder (IEDDA) reaction as part of a "click to release" technique(Wang,et al., 2019)

Alt Text for the figure: The 1, 4-dihydropyridazine intermediate 22 can either be directly eliminated or undergo tautomerization and 1, 4-elimination to liberate 25 and the payload molecule R1NH2 following the cyclization between TCO (20) and tetrazine (21).

2.2. Bioorthogonal shear reactions

Corresponding to the bioorthogonal coupling reaction dominated by bonding chemistry, the bioorthogonal shearing reaction is to achieve the in-situ release, activation or functional regulation of the target biomolecule by means of chemical bond cleavage. Since its proposal, bioorthogonal shear reaction has shown broad application prospects and potential, and has attracted more and more attention.

2.2.1. Light-mediated shear reaction

Most of the earliest shear reactions discovered and applied in the field of chemical biology are based on light-mediated deprotection reactions such as o-nitro benzyl (ONB) derivatives. However, these reactions have limitations due to problems such as phototoxicity and low tissue penetration, as well as incomplete biorthogonality.

2.2.2. Metal-mediated shear reactions

A metallic Ru-mediated desallyl oxycarbonyl (allyl carbamate) reaction in living cells was initially documented by Meggers et al. in 2006, and used this reaction to activate small molecule fluorescent dyes in living cells (Meggers, et al., 2006). Since then, transition metal-mediated bioorthogonal shear reactions have been widely studied, and the types of transition metals mediating bond breaking have been enriched and expanded. Some of the more important metal-mediated shear reactions that have been identified since then include: In 2013, Finn's group reported a copper-mediated shear reaction of dimethyl-substituted alkynyl propoxycarbonyl group (Finn,2013). In 2017, the Unciti-Broceta group reported a gold-mediated shear reaction of alkynyl propoxycarbonyl (Unciti-Broceta, 2017).

2.2.3. Chemical small molecule-mediated shear reactions

In 2008, Fox's group reported the rapid coupling reaction of cyclic tension trans-cyclooctene pro-dienophiles with tetrazine compounds (diens) under biocompatible conditions (Blackman et al., 2008). Around the same time, Hilderbrand's group reported the coupling reaction of norbornene with tetrazine and applied it to the fluorescent labeling of biological macromolecules (Hilderbrand, 2008). Since then, the research and exploration of IEDDA as a biological orthogonal coupling reaction has begun. In recent years, based on the characteristics of flexible structure-activity of chemical small molecules and ease of penetrating into the tissue, IEDDA-mediated biorthogonal shearing reactions have great advantages. Based on the efficient, fast and orthogonal characteristics of this reaction, the rapid activation of enzymes can be achieved in living cells.

3. The application of click chemistry and gene editing technology in tumor targeting research

3.1. A rapid site-specific labeling method for proteins based on IEDDA bioorthogonal reactions

The specific insertion of high-tension alkenes or alkynes into the protein of interest by genetically encoded means, the use of fluorescent probes with tetrazine can be used for rapid labeling, especially the insertion of trans-cyclooctene into the protein, and the labeling reaction can achieve a rate of antibody-antigen complex formation comparable to that of the currently commonly used antibody labeling methods (kasso=0.1~3*106 M-1 •s-1). Proteins on the surface and inside living cells can be labeled using this technique, however tetrazine probe stability in vivo needs to be increased, and the above labeling reactions at the level of living animals need to be explored (Wang&Ye, 2012). In 2024, the team of Professor Liu Tao develop a new five-membered diheterocyclic monosubstituted tetrazine that is a very beneficial addition to the biorthogonal toolkit, especially the imidazole tetrazine molecule, which has both a very high reaction rate and good stability, so that it can be genetically encoded into the cell's proteome, becoming the fastest site-directed biorthogonal tag in eukaryotic cells (Liu, 2024).

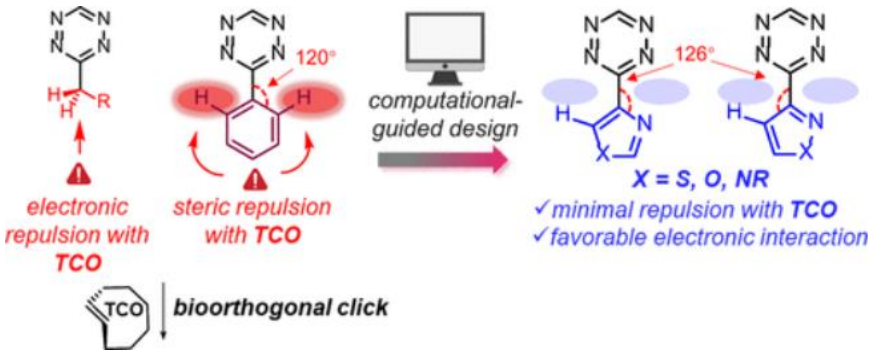

Figure 2. Computationally Guided Design for BOC Reactions (Liu, 2024)

Alt Text for the figure: Through theoretical calculations that both types of commonly used monosubstituted tetrazine molecules have their own defects, and finally proposed that the five-membered diheterocyclic monosubstituted tetrazine molecules are potentially better.

As shown in figure 2, the newly discovered novel quinnary diheterocyclic monosubstituted tetrazine can overcome the shortcomings of the poor stability of tetrazine probes in Because of its incredibly quick reaction rate and high stability, and realize an efficient protein anchoring technology.

This rapid site-specific labeling approach can provide a very useful tool for targeted tumor therapy by labeling polysaccharides, glycoproteins, or proteases on tumor cell membranes and subsequently targeting conjugated molecular carriers in tumor therapy.

3.2. BOC chemistry makes up for the shortcomings of gene editing technology in tumor targeted therapy

Tumor-specific T cell therapy has limited efficacy in solid tumors, mainly due to the loss of metabolic function of gene-edited immune cells. Hao et al. applied a click reaction to click on the surface of T cells and anchor the cholesterol acyltransferase inhibitor avatimibe, which can increase cholesterol on the T cell membrane, thereby activating T cells and enhancing their tumor-killing effects. This strategy of reconstitution Ing T cell metabolism through click chemistry enhances the function of adaptive T cell therapy (Hao, et al., 2020).

As a bispecific antibody, the T cell engager can link tumor-associated antigens and T cell antigens, and the bispecific T cell engager modified by the click chemotherapy method to modify the synthesis of IgG1 antibody exhibits tumor-killing activity at picomolar concentration. Because chimeric antigen receptor (CAR) T cells cannot enter the tumor body or the immunosuppressive tumor microenvironment (TME), they have a limited therapeutic effect on solid malignancies. By editing the surface of CAR-T cells with hyaluronidase and the inhibitory checkpoint antibody α-PDL1, Zhao et al. improved the therapeutic impact of CAR-T cells on solid malignancies using BOC chemistry (Zhao, et al., 2022). The modified hyaluronidase is degraded to hyaluronic acid, which encourages the infiltration of CAR-T cells into the tumor body and eliminates the extracellular matrix of the tumor, while the cells edited with α-PDL1 also have stronger antitumor activity than traditional CAR-T cells. Crucially, in solid tumor models, CAR-T cells modified with hyaluronidase and α-PDL1 demonstrated good therapeutic activity without any notable toxic side effects (Zhang&Yue, 2024).

3.3. Construction of bispecific antibody platform

3.3.1. Antibody-CRISPR/Cas conjugation technology for gene editing and delivery to particular cancer targets

HER2-positive malignancies can benefit from targeted gene editing and targeted delivery thanks to an antibody-CRISPR/Cas conjugate technology. The design of CRISPR/Cas systems involves replacing particular Cas9 residues with synthetic amino acids that can form complexes with nanocarriers and be bioorthogonally functionalized using HER2-targeting monoclonal antibodies. In vitro, HER2-positive cancer cells can be selectively targeted for gene editing by the resultant antibody-conjugated CRISPR/Cas nanocomplexes. Research has demonstrated that the in vivo administration of antibody-CRISPR/Cas nanocomplexes can considerably slow the growth of tumors by efficiently disrupting the plk1 gene in HER2-positive ovarian cancer. For the treatment of different malignancies and genetic disorders, the current study offers a practical therapeutic platform for antibody-mediated CRISPR/Cas delivery (Chung, et al., 2024).

3.3.2. Delivery of the CRISPR/Cas9 system to specific tumors via bioorthogonal response for dual-targeted cancer immunotherapy

A new CRISPR/Cas9 tumor targeted delivery method based on biorthogonal processes for dual-target cancer immunotherapy was investigated and put out by Prof. Yang's group in July 2023. First, the TME-biodegradable hollow manganese dioxide (H-MnO)2) nanoplatform simultaneously activates the cGAS-STING pathway and selectively in vivo metabolic labeling of cancer. Protein

tyrosine phosphatase N2 (PTPN2) is then lost as a result of the accumulation of CRISPR/Cas9 system-loaded liposomes within the altered tumor tissue by in vivo click chemistry, further sensitizing the tumor to immunotherapy. Overall, by strengthening innate and adaptive anti-tumor immunity, our approach demonstrates strong anti-tumor responses and offers a modular platform for in vivo precise gene editing (Yang, 2023).

3.4. BOC chemistry activates gene editors mediating pyroptosis in tumor therapy

Peng Chen/Xinyuan Fan's team further applied the IEDDA biorthogonal shearing reaction to the chemical regulation of the protein-DNA interface, using tetraazine-triggered trans cyclooctene removal to realize the regulation of Cas9 protein and PAM sequence binding, and then developed APOBEC1 a chemically controllable C-to-T base editing technology BaseBAC (Bioorthogonally Activatable Base Editor) in living cells by fusing deaminases. It is used for in situ regulation of self-inhibitory C-terminal expression of GSDME proteins (CAA-to-TAA base editing at position Q287) for chemical regulation of pyroptosis (Cheng&Fan,2022). The specific approach is shown in figure 3. This technology can be further applied to tumor treatment to induce pyroptosis of tumors to achieve the therapeutic purpose.

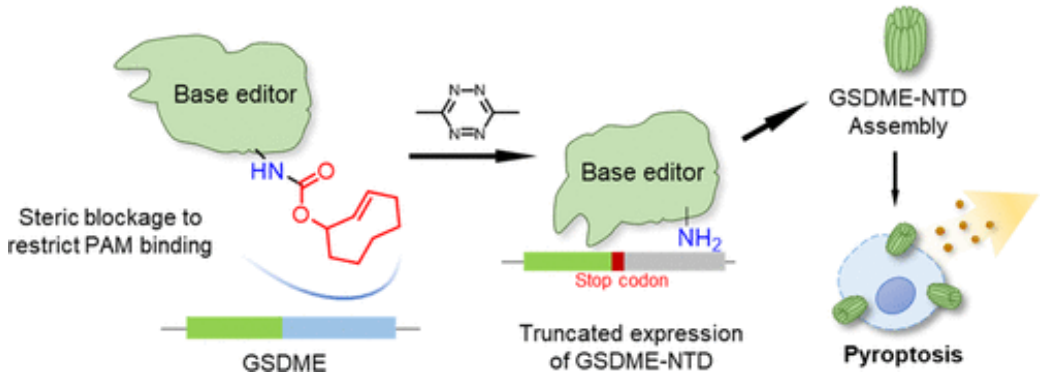

Figure 3. Design of a Bioorthogonally Activatable Base Editor for Gasdermin's Functional Awakening (BaseBAC) (Cheng&Fan, 2022).

Alt Text for the figure: The experiment initially established a bioorthogonal blockade on the PAM-interacting residue to limit its DNA-binding ability, thereby making the enzymatic activity of a cytosine base editor (CBE) switchable. In order to trigger pyroptosis, the resulting BaseBAC enabled in situ control of base editing on the GSDME gene, which changed to the shortened expression of its N-terminal domain.

4. Non-small molecule-mediated bioorthogonal reactions are applied to tumor therapy

4.1. Light-mediated bioorthogonal shearing response is applied to tumor therapy

Zhibo Liu's group has developed a radiation-driven biological orthogonal shear reaction. By designing dihydroxybenzyl carbonate as a chemoprotective group, they are able to react with the hydroxyl radicals generated by external radiation and then efficiently and selectively remove them through 1, 4 or 1, 6 eliminations, thereby releasing the target molecule, to achieve the release of fluorescent molecules in tumor cells and living tissues (Liu, 2020). This method has high spatiotemporal resolution and high tissue penetration ability, which is expected to achieve the precise release of chemotherapy drugs driven by exogenous radiation therapy, and then bring a new dawn to cancer treatment.

4.2. Metal-mediated bioorthogonal shearing reaction is applied to tumor therapy

By designing a functionalized palladium catalyst to target aggregation in tumor cells with high integrin expression, and further skillfully using palladium-mediated trans metallization to activate

stable nitrogen heterocyclic carbene-gold complexes to generate active gold species with high cytotoxicity, thereby inducing anticancer activity in cells and in vivo. This method can effectively avoid the competitive binding of gold complexes with antitumor activity to sulfhydryl compounds in vivo, and at the same time, the spatiotemporal-specific selectivity realizes its controllable activation in biological systems, which provides a new strategy for biological orthogonal activation. Based on amino and phenolic hydroxyl groups, Chen's group created and manufactured controlled-release antibody-drug conjugates (Cleavable ADCs), which effectively killed cancer cells selectively (Reetz, et al., 2020).

5. Conclusion

In this research report, the application potential of biorthogonal response-assisted gene editing technology in tumor treatment is deeply discussed through the introduction of biorthogonal response and the advantages of its application in tumor treatment. Through a series of innovative experimental design and research methods, the combination strategy has successfully demonstrated the significant advantages of enhancing the treatment effect of tumors, improving the precision of treatment, and reducing side effects.

In summary, biorthogonal combined with gene editing technology provides a new idea and method for tumor treatment. Because of its incredibly quick reaction rate and high stability, the newly discovered pentavariant diheterocyclic monosubstituted tetrazine can be considered in combination with gene editing technology for protein anchoring, controllable activation of T cell epitopes, construction of modular platforms or participation in the regulation of base editors in the future to achieve the purpose of tumor treatment.

References

[1] Blackman, M. L., Royzen, M., & Fox, J. M. 2008. Tetrazine ligation: fast bioconjugation based on in-verse-electron-demand Diels− Alder reactivity. Journal of the American Chemical Society, 130 (41), 13518 - 13519.

[2] Devaraj, N. K., Weissleder, R., & Hilderbrand, S. A. 2008. Tetrazine-based cycloadditions: application to pretargeted live cell imaging. Bioconjugate chemistry, 19 (12), 2297 - 2299.

[3] Hao, M., Hou, S., Li, W., Li, K., Xue, L., Hu, Q., ... & Zhang, C. 2020. Combination of metabolic in-tervention and T cell therapy enhances solid tumor immunotherapy. Science translational medi-cine, 12 (571), eaaz6667.

[4] Ji, X., Pan, Z., Yu, B., De La Cruz, L. K., Zheng, Y., Ke, B., & Wang, B. 2019. Click and release: bioorthogonal approaches to "on-demand" activation of prodrugs. Chemical Society Reviews, 48(4), 1077-1094.Streu, C.; Meggers, E. Angew. Chem., Int. Ed. 2006, 45, 5645.

[5] Kislukhin, A. A., Hong, V. P., Breitenkamp, K. E., & Finn, M. G. 2013. Relative performance of al-kynes in copper-catalyzed azide–alkyne cycloaddition. Bioconjugate chemistry, 24(4), 684-689.

[6] Li, Y., Su, Y., Wang, H., Xie, Y., Wang, X., Chang, L., ... & Liu, T. 2024. Computation-Guided Dis-covery of Diazole Monosubstituted Tetrazines as Optimal Bioorthogonal Tools. Journal of the Amer-ican Chemical Society, 146 (39), 26884 - 26896.

[7] Ngai, W. S. C., Yang, S., Zeng, X., Liu, Y., Lin, F., Wang, X., ... & Chen, P. R. 2022. Bioorthogonally activatable base editing for on-demand pyroptosis. Journal of the American Chemical Socie-ty, 144 (12), 5411 - 5417.

[8] Pérez-López, A. M., Rubio-Ruiz, B., Sebastián, V., Hamilton, L., Adam, C., Bray, T. L., ... & Unciti-Broceta, A. 2017. Gold-triggered uncaging chemistry in living systems. Angewandte Chemie Inter-national Edition, 56 (41), 12548 - 12552.

[9] Qu, G., Li, A., Acevedo-Rocha, C. G., Sun, Z., & Reetz, M. T. 2020. The crucial role of methodology development in directed evolution of selective enzymes. Angewandte Chemie International Edi-tion, 59 (32), 13204 - 13231.

[10] Saxon, E., & Bertozzi, C. R. 2000. Cell surface engineering by a modified Staudinger reac-tion. Science, 287 (5460), 2007 - 2010.

[11] Streu, C., & Meggers, E. 2006. Ruthenium-induced allylcarbamate cleavage in living cells. ANGEWANDTE CHEMIE-INTERNATIONAL EDITION IN ENGLISH-, 45 (34), 5645.

[12] Versteegen, R. M., Rossin, R., ten Hoeve, W., Janssen, H. M., & Robillard, M. S. 2013. Click to re-lease: instantaneous doxorubicin elimination upon tetrazine ligation. Angewandte Chemie Interna-tional Edition, 52 (52), 14112 - 14116.

[13] Wang, Q., Wang, Y., Ding, J., Wang, C., Zhou, X., Gao, W., ... & Liu, Z. 2020. A bioorthogonal system reveals antitumour immune function of pyroptosis. Nature, 579 (7799), 421 - 426.

[14] Wang Xin, Zhang Xianrui, Huang Zongyu, Fan Xinyuan, & Chen Peng. 2021. Research progress of bioorthogonal reactions in China. Acta Chimica Sinica, 79 (4), 406.

[15] Wang Yue, & Ye Xinshan. 2012. A rapid site-specific protein labeling method based on Diels-Alder bioorthogonal reactions. Acta Chimica Sinica, 70, 2208 - 2212.

[16] Yang, J., Yang, K., Du, S., Luo, W., Wang, C., Liu, H., ... & Song, Y. 2023. Bioorthogonal Reaction-Mediated Tumor-Selective Delivery of CRISPR/Cas9 System for Dual-Targeted Cancer Immunother-apy. Angewandte Chemie, 135 (37), e202306863.

[17] Yang, S., Im, S. H., Chung, J. Y., Lee, J., Lee, K. H., Kang, Y. K., & Chung, H. J. 2024. An Antibody-CRISPR/Cas Conjugate Platform for Target-Specific Delivery and Gene Editing in Cancer. Advanced Science, 2308763.

[18] Zhao, Y., Dong, Y., Yang, S., Tu, Y., Wang, C., Li, J., ... & Lian, Z. 2022. Bioorthogonal equipping CAR-T cells with hyaluronidase and checkpoint blocking antibody for enhanced solid tumor immu-notherapy. ACS Central Science, 8 (5), 603 - 614.

[19] Zhang Baihong, & Yue Hongyun. 2024. The application of click chemistry in tumor research. Cancer Prevention Research, 51 (11), 951 - 955.