CRIPS Digest

Semaglutide: GLP-1 agonist in the treatment of Non-alcoholic Steatohepatitis

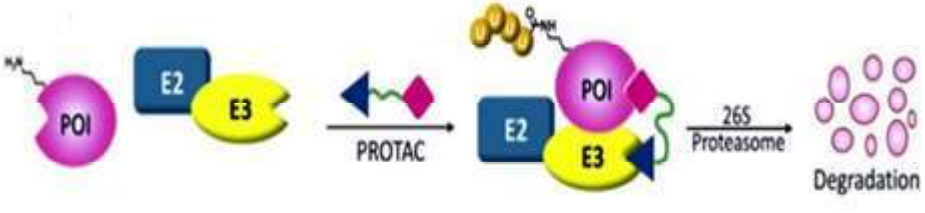
Semaglutide, a potent GLP-1 agonist which has been approved for the treatment of type 2 diabetes mellitus, recently showed protective effect against Non-alcoholic Steatohepatitis (NASH). It is a longer acting GLP-1 receptor agonist with 94% structural similarity with native USFDA approved GLP-1 agonists which are available as Ozempic (s.c injection, weekly once dosing with 0.5 and 1 mg strength) and Rybelsus (oral tablet, once daily dosing in 3, 7 and 14 mg strength). NASH is associated with disruption in insulin signaling, lipid peroxidation, activation of inflammatory pathways and liver injuries and Semaglutide shows indirect protective effect through gut-pancreases-liver axis. This drug showed protective role by modulating hepatic mitochondrial function, increasing insulin sensitivity and reducing accumulation of free fatty acid. In a phase 2 clinical trial, Semaglutide (s.c, daily dose of 0.1, 0.2 and 0.4 mg strength) improved the NASH by 40%, 30% and 15% respectively as compared to the placebo and even without worsening the fibrosis. In other placebo-controlled phase 2 study, similar Semaglutide (given in s.c weekly once dose) in 65 subjects and on MRI study it shows protective effect by ameliorating the liver fibrosis, change in liver stiffness and liver fat content. In a phase-3 clinical trial Semaglutide improved cirrhosis, resolution of steatohepatitis and histology of balloon shaped hepatocyte in NAH patients, however without cirrhosis. So Semaglutide can be given in patient as monotherapy or along with dietary restriction and exercise for the treatment of NASH. (Nat. Rev. Immun., (2022) 22: 429-443)

Discovery of Pyrrolopyrimidine Derivatives as Selective Perinucleolar Compartment (PNC) inhibitors, a Phenotypic Marker of Tumor Progression

Cancer, one of the major health problems and second leading cause of death globally is still a serious threat to human health worldwide, despite the development of various treatments. It involves a process by which primary tumors disseminate throughout the body to secondary sites, which happens to be the foremost cause of mortality for >90% of cancer patients. To overcome this, a phenotypic marker perinucleolar compartment (PNC), which can identify cancer cells competent to metastasize and correlate with cancer progression and metastatic capacity, make it a useful marker for metastatic cancer progression. The PNC is a membrane-less, highly dynamic subnuclear body found at the periphery of the nucleolus. It is enriched with RNA transcripts and RNA-binding proteins, reflecting different states of genome organization. It forms only in cancer cells but not in normal, non-transformed cells including embryonic stem cells. The authors carried out the work identifying and developing novel compounds that reduce PNC prevalence at concentrations where cell viability is not affected. They performed detailed medicinal chemistry optimization campaign around a pyrrolopyrimidine series that ultimately led to the discovery of the bioavailable analogue, metarrestin (NCATS-SM0590), which has shown potent antimetastatic activity with improved survival in rodent models and is currently being evaluated in a first-in-human phase 1 clinical trial. (J. Med. Chem. 2022, 65, 8303; Sci. Transl. Med. 2018, 10, 8307.).

PROTAC as heterobifunctional modality in drug discovery

PROTACs or proteolysis targeting chimeras is a strategy that utilizes ubiquitin proteosome system to target a specific protein which lead to its degradation in the cell. PROTACs are hetero bifunctional molecules that connect a protein of interest ligand to an E3 ubiquitin ligase (E3) recruiting ligand with an optimal linker. Nowadays PROTAC technology is used to target varieties of proteins including transcription factors, skeleton proteins, enzymes, and regulatory proteins. This technology is used to treat various diseases like cancer, viral infections, immune disorders and neurodegenerative disorders. This is mainly due to the potent ability of PROTACS to induce targeted protein degradation



by using body's ubiquitin proteasome system. It is not possible to develop drugs or viable small molecules that act on undruggable targets like transcription factors, IKZF1/3, CSNK1A1, ZFP91 that inhibit interactions of endogenous proteins or nucleic acids on these targets. By using PROTACs we are artificially mimicking this process to carry out degradation of desired protein.

Steps involved in PROTAC technology

1. The PROTAC binds both the target protein and the E3 ligase simultaneously to induce the formation of a ternary complex.
2. The target protein is then polyubiquitinated by E3 ligase.
3. Proteolysis of protein of interest by 26S Proteasome.

There are different PROTACs based on the different types of E3 ubiquitine ligases like ring family E3s, Culling ring E3s, Von Hippel-Lindau, cereblon (CRBN), Cellular Inhibitor of apoptosis (cIAP) and Mouse Double Minute 2 homolog (MDM2).

ARV 110 and ARV 471 are in clinical trial phase ?? for the treatment of prostrate cancer and breast cancer respectively. (Nat. Rev. Drug Discovery. 2022, 21, 3, 181-200; J. Med. Chem. 2018, 61, 2, 444-452.)

Programmed genome editing by a miniature CRISPR-Cas12f nuclease.

The CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeat- CRISPR-associated protein) system has been employed as a system of adaptive immunity in bacteria and archae. It found increasing use as a gene editing tool in laboratory as well as clinical setting. However, their large size makes their encapsulation in vectors with limited size difficult. Wu et al. report the characterization of Acidibacillus sulfuroxidans Cas12f1 (AsCas12f1) (422 amino acids), an example of a miniature type V-F effector protein. Type V-F class (Cas12f) is characterized by small Cas effector proteins (400-700 residues). The protospacer adjacent motifs (PAM) specificity of CRISPR-AsCas12f1 was determined by subtractive high throughput screening of an E. coli library of randomized PAM sequences. PAM depletion assay showed that AsCas12f1 effector recognized PAMs as 5?-NTTR (R: A/G). The RNA component of AsCas12f1 was identified by small RNA seq to be mature crRNA (45-48 nt) and probable tracrRNA (138-144 nt). Fluorescence labelling revealed the putative dsDNA substrate which was degraded with the generation of 11 nt 5? overhang at target strand near the PAM. A non-cohesive end was generated at the segment away from the protospacer. AsCas12f1 also recognized ssDNA and cleaved it at the same site as dsDNA but this effect was not dependent on PAM. The ability of the screened Cas effector to carry out gene manipulation in bacteria was monitored in K. pneumoniae. Both drug-resistant and metabolic genes could be disrupted. AsCas12f1 was also able to accomplish gene editing in HEK293 cells where an inactivated EGFP expression cassette could be activated by restoration of the reading frame. AsCas12f1 is the smallest programmable endonuclease currently in use for human cell editing and allows its packaging into adeno-associated viral vector. The encapsulated CRISPR-AsCas12f1 system was able to edit targeted genes in different cell lines. (Wu Z, Zhang Y, Yu H, Pan D, Wang Y, Wang Y, Li F, Liu C, Nan H, Chen W, Ji Q. Programmed genome editing by a miniature CRISPR-Cas12f nuclease. (Nat. Chem. Biol. 2021, 17, 1132-1138).