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WHAT ARE THE DRUG CANDIDATES THAT IRBM IS CURRENTLY DEVELOPING?

Our peptide chemistry team has extensive expertise in peptide and macrocyclic drug discovery in various therapeutic areas and a track record in the clinic. Drug candidates that we are currently developing include peptides that incorporate non-proteinogenic amino acids and peptide conjugates to various pharmacokinetic enhancing moieties such as fatty acids, cholesterol and polymers that enable prolonged *in vivo* exposure due to reduced renal filtration and proteolysis. Much emphasis is on the stabilization of our leads by metabolite identification from high resolution mass spectrometry studies on *in vitro* and *in vivo* samples. Our drug candidates also include a very important class of molecules specifically peptide macrocycles of various chemical complexity that can incorporate multiple chemical constraints and non-natural building blocks.

ARE PEPTIDE MACROCYCLES PROMISING DRUGS CANDIDATES? WHY?

Nowadays, there is a strong drive for chemists to explore new areas of chemical space in search of innovative ways to bind and modulate challenging biological targets. In the past few years, there has been an increased interest in exploring larger (700–1900 Da) macrocyclic compounds as a new modality to address undruggable biological targets such as protein–protein interactions (PPIs), including intracellular targets. Macrocycles have proven to be very valuable tools in the chemical space beyond the conventional drug-like small molecules and the biological drugs such as monoclonal antibodies. To this purpose novel screening techniques using powerful in vitro display systems have emerged that can provide useful macrocyclic peptide leads capable of selective binding to relatively shallow protein surfaces often involved in clinically important protein-protein interactions. That's why, in the last few years, we, at IRBM, have been focusing on this chemical space and our team has acquired a broad knowledge of macrocyclic peptide synthesis across different therapeutic areas. Macrocyclization restricts the conformational flexibility of a peptide to a subset of structures sampled by the linear form, and can effectively pre-organize a larger compound for target binding. As a result of this pre-organization, the entropic penalty for target binding is decreased and in some cases cyclization can lead to an increase in target affinity and specificity. The cyclic nature of this kind of peptides enhances their stability against proteolytic degradation by peptidases and potentially, after extensive optimization predisposing to cellular permeability and oral bioavailability.

WHICH CHEMICAL APPROACHES HAS IRBM EMPLOYED FOR PEPTIDE AND MACROCYCLIC DRUG OPTIMIZATION?

For the optimization of peptide and macrocycle leads we harness our expertise in solution and solid phase chemistry and also in chemo-selective conjugation approaches. The chemical strategies for peptide and macrocyclic drug optimization that we employ include multi-analogue synthesis taking advantage of a wide in-house collection of non-proteinogenic amino acids.

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Director Peptide Chemistry at IRBM S.p.A.

In addition, we routinely perform ad-hoc synthesis of novel amino acids and building blocks by organic synthesis in order to further expand structure activity relationship studies and intellectual property coverage. There is also a special emphasis on the application of various cyclization chemistries in our leads to improve various drug-like features such as stability, potency and potentially permeability and oral bioavailability.
With the assistance of many high throughput microwave automated synthesizers, we are capable of increasing chemical coupling efficiency and reducing synthesis time by 90% on average. Chemical strategies are employed to increase the quality of the crude material enabling us to synthesize complex peptides >100 residues in a reliable fashion with an additional positive impact on the purification yield.
We also approach the synthesis of longer peptides and small protein domains by native chemical ligation as an alternative chemo-selective peptide conjugation technique to non-native methods. This strategy can provide discrete coupling of the N-terminus of one peptide to the C-terminus of another peptide using a unique reaction process which essentially extends the peptide chain while maintaining native sequence and bonding characteristics.

COULD YOU BRIEFLY DESCRIBE THE WORK FLOW FOR PEPTIDE DISCOVERY IN IRBM?

Our general work flow for peptide drug development starts with extensive design and engineering involving the optimization of multiple parameters. We have an integrated platform at IRBM to support peptide drug discovery that spans several different but complementary expertise. Our discovery teams can take advantage of various technologies, including high-throughput and high-content screening capabilities, CADD (computer-aided drug design), phage display, CD and NMR spectroscopies to optimize activity, efficacy and selectivity. We improve pharmacokinetic profiles in order to overcome rapid metabolism and disposition due to enzymatic degradation and renal filtration. Our ADME/DMPK group has remarkable expertise in identifying peptide metabolites from *in vivo* and *in vitro* samples using top-notch high resolution MS technologies. As an example, innovative in *vitro* approaches were recently implemented to assess stability of therapeutic peptides in subcutaneous tissue in addition to the more routine plasma/blood matrix investigations. We work very carefully to identify spots of chemical instability and to study aggregation and oligomerization using LC-MS and NMR.
Early in the drug discovery process we screen for potential hypersensitivity reactions including anaphylaxis that have been reported as a significant issue for some approved peptide drugs. Some of these reactions can occur at first administration without prior exposure, especially for the sub-cutaneous route, suggesting a non IgE-mediated hypersensitivity reaction. The mechanism underlying this adverse and off-target effect mostly seen in NHP and humans is due to a receptor-mediated mast cell activation with consequent histamine release. Therefore, to de-risk our discovery programs and prioritize leads, early in each program we also screen for potential pseudo-allergic reactions caused by lead peptides using the hLAD2 mast cell line for which we obtained a license from NIH.