Triad Therapeutics: integration of NMR structural determinations and smart chemistry to speed drug discovery

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Triad Therapeutics' proprietary technologies enable dramatic increases in both the speed of lead compound generation and the quality (binding affinity and specificity) of the compounds produced. Triad's approach, called Integrated Object-oriented PharmacoEngineering (IOPE™), focuses on designing inhibitors for entire protein families, such as oxidoreductases and kinases, thereby enabling parallel discovery of inhibitors for multiple targets within a protein family. The technology uses nuclear magnetic resonance (NMR) spectroscopy-based structural information to drive inhibitor design without the need for full structures, therefore significantly expediting the drug discovery process.

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▼ Genomic and proteomic research has recently discovered a plethora of drugable protein targets [1]. Although genomics and proteomics have fostered the elucidation of protein function, they have not changed the fundamental quest for highly specific, high-affinity inhibitors. In fact, the abundance of new target proteins has only increased the need for technologies to design drug-like inhibitors more quickly. Triad (Box 1) seeks to ease the bottleneck caused by too many targets and too few leads by providing specific and high-affinity lead molecules using an integrated mix of smart chemistry and structural biology.

Recently, two approaches, namely combinatorial chemistry and structure-based drug design, have been brought to bear on this challenge, but neither has yet fulfilled its promise. Combinatorial chemistry has yielded lower than expected results because of the very large number of possible chemical entities, the majority of which are not appropriate drug candidates. Meanwhile, structure-based design has been hampered by slow throughput caused by the need to solve whole protein structures. Triad has therefore developed a suite of technologies that create small, focussed libraries, and avoid the time-consuming need for complete structural determination by using proprietary and fast nuclear magnetic resonance (NMR) techniques, innovative bioinformatics and smart chemistry.

Proteomic leverage and smart chemistry

Triad focuses on large protein families, such as oxidoreductases and kinases, rather than on individual target proteins. This approach leverages the observation that certain ligand binding sites are conserved across a large number of protein targets and, as such, serve as common starting points for drug design for an entire gene family. Ligands are designed from existing genomic and proteomic data to bind to all members of a protein family in highly conserved cofactor binding domains [such as the NAD(P) binding site in oxidoreductases]. This approach enables rapid progress from targets within these protein families to high-affinity lead compounds in a time frame of weeks rather than the traditional period of years (Fig. 1).

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Box 1. Triad Therapeutics: company information

- Triad began operation in mid-1999 with the aim of addressing the critical shortfall in lead compounds being developed against the increased number of targets generated by the genomics revolution.
- Triad has had two rounds of financing raising US$42.5 million in total.
- Triad employs 50 scientists (27 with PhDs) among its staff of 60 people.
- Triad has assembled a multidisciplinary team with strong backing from knowledgeable investors and from its Scientific Advisory Board (see Box 2).
- Triad plans to occupy a new, specially designed 50,000 ft² facility in March 2002 in the La Jolla financial district of San Diego, CA, USA.

Bioinformatics

First, a given gene family that contains a common binding site is divided into subgroups of related binding sites termed pharmacofamilies. Pharmacofamilies are characterized by cofactor binding sites with shared, highly conserved electrostatic properties and shapes (Fig. 1a). For example, analysis of oxidoreductases yields a finite number of ~10 pharmacofamilies. Parsing gene families into meaningful pharmacofamilies has required the development and implementation of novel and proprietary bioinformatics tools.

Discovering common ligand mimics

Triad then identifies drug-like molecules [common ligand mimics (CLMs)] that fit into the common binding site shared by a pharmacofamily (Fig. 1b). These CLMs require only modest potency for the target and have been identified by both enzymatic screening and virtual screening for all members of a protein pharmacofamily.

NMR structural information

Triad's proprietary NMR technology is then used to glean two pieces of cogent structural information [2]. First, the CLM binding orientation in the active site is determined to ensure that it is appropriate and, second, the best position to add a chemical linker that connects to a 'specificity ligand' is determined (Fig. 1c). It is important to note that although the availability of the complete structure of the target facilitates these approaches, such data are not required. Moreover, Triad's proprietary NMR technologies require the use of labeled protein only in certain instances and are not limited by the molecular weight of the protein target. These factors, plus the fast turnaround time typical of Triad's NMR experiments (minutes to several days) differentiate Triad's NMR platform from other available structure-based technologies that generally take several months, and lack proteomic leverage.

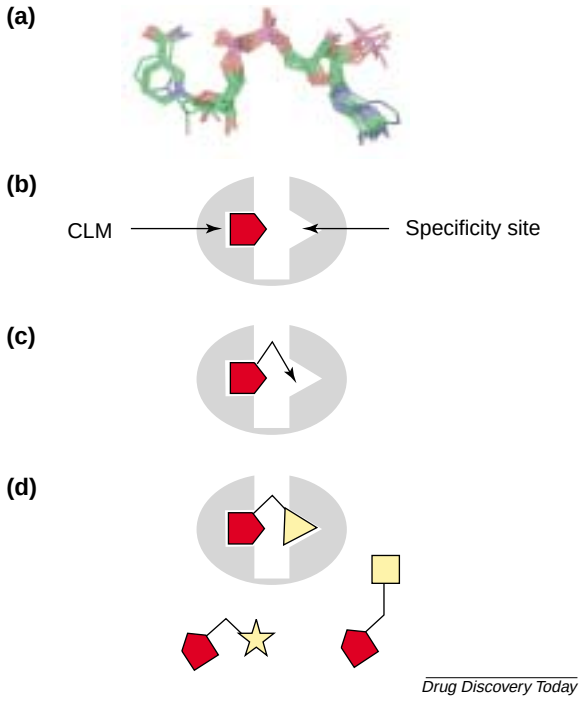

Figure 1. Integrated Object-oriented PharmacoEngineering (IOPE™) technology comprises four components. **(a)** Parsing gene families into homogeneous subfamilies (pharmacofamilies). The geometry of the cofactor NAD(P) in the binding site of eight members of a single oxidoreductase pharmacofamily is shown. **(b)** Discovery of common ligand mimics (CLMs) for common ligands (such as NADH or ATP). The bound CLM is shown as a red pentagon. **(c)** Use of fast nuclear magnetic resonance (NMR)-derived structural information to define the best site on the CLM to add linker. The linker is shown off the appropriate point on the CLM. **(d)** Construction and screening of bi-ligand libraries (CLM and specificity site ligands). Several representative bi-ligand members of a library are shown using the CLM with different specificity ligands (SLs) (triangle, rectangle, star). Note that the bi-ligand with the triangle is bound as an inhibitor.

Specificity ligands (SLs) are then added to the distal site on the CLM's linker. The SLs act as a second binding ligand, increasing binding affinity and adding specificity. As with CLMs, the SLs alone need only bind with modest affinity. Although the SLs might appear to be largely random, Triad selects the SLs by considering the structural diversity of the natural substrates of the enzyme. Like CLMs, SLs are designed to be drug-like and to maximize the bioavailability of the resultant bi-ligand (CLM + SLs) libraries (Fig. 1d). Thus, a few CLMs are combined with thousands of SLs to produce a library of thousands of bi-ligand molecules.

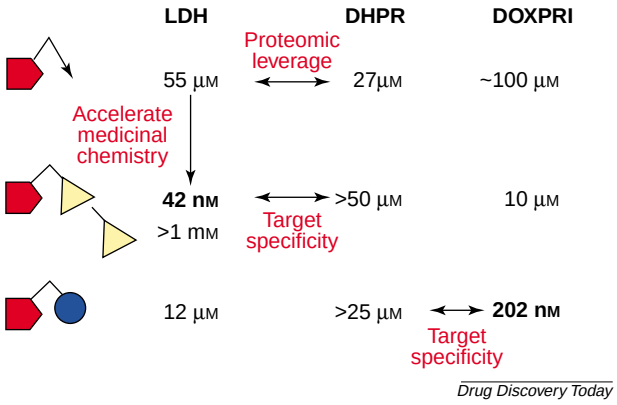

Figure 2. Identification of specific nanomolar leads across the oxidoreductase gene family from a small bi-ligand library. A common ligand mimic (CLM) (red pentagon) plus linker that bound weakly to three oxidoreductases [lactate dehydrogenase (LDH), dihydrodipicolinate reductase (DHPR) and 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DOXPRI)] was used to build a small library with 300 specificity ligands (SLs). The library was screened against the panel and different nanomolar hits (42 nM versus LDH and 202 nM versus DOXPRI) using unique SLs (yellow triangle versus blue circle) were discovered. When tested alone, the LDH SL (yellow triangle) bound to LDH at >1 mM, showing the power of bi-ligand inhibitors. This figure illustrates that using a CLM gives proteomic leverage over a gene family, and the addition of SLs provides specificity and increased binding energy to accelerate medicinal chemistry on the molecules.

Proof of concept

Using this approach, Triad has recently built one small-scale library (built only with binding-site structural information) based on a single CLM and only 300 SLs. Screening three oxidoreductase targets against this mini-library enabled the discovery of several bi-ligand nanomolar inhibitors (Fig. 2). This impressive result began with a CLM that bound modestly to each of three oxidoreductase enzymes at 40–200 μM. Specific inhibitors were found for two of the enzymes, lactate dehydrogenase (LDH) and 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DOXPRI), the latter of which has no extant structural information (the x-ray crystallographic structure appeared after submission of this article). These specific inhibitors show a 500–1000-fold increase in binding affinities when compared with the CLM alone (Fig. 2). The mini-library built for our proof-of-principle experiments contained only ~300 SL moieties. With such a limited breadth of coverage, we expected not to get full oxidoreductase family coverage. We have subsequently taken a known DHPR specificity site inhibitor [pyridine dicarboxylate (PDC)] and attached it to several of our drug-like CLMs. This resulted in a bi-ligand inhibitor of DHPR with a K_i of 110 nM.

Ongoing and future projects

Triad has since completed construction of a library of bi-ligands using three drug-like CLMs for the oxidoreductase

Box 2. Triad's investors and key personnel[a]

Board of directors
John Freund (Skyline)
Yasunori Kaneko (Skyline)
Martial Lacroix (Genechem)
Paul Brooke (MSDW)
Michael Meyers (Global Biomed.)
Daniel Petree (P^2 Partners)
Stephen Coutts (Triad)

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Skyline Ventures
CSFB Private Equity
Intl Biomedicine Holdings
Hambrecht and Quist
J & J Development Corp.
Lombard Odier
Mediphase Venture Partners
Genechem Tech. Venture Fund

Key Executives
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Christine Gray-Smith, Chief Financial Officer
Joel Smith, Executive Director, Business Development
Rick Jack[b], Vice President, Biology
Daniel Sem[b], Vice President, Biophysics
Hugo Villar, Vice President, Computational Biophysics
Lin Yu[b], Vice President, Chemistry

Scientific advisory board
W.W. Cleland (University of Wisconsin)
Barry Honig (Columbia University)
Amir Hoveyda (Boston College)
Chris Raetz (Duke University)
Kurt Wuthrich (ETH Zurich and Scripps)

[a]Please refer to www.triadt.com for biographies of these and other key personnel.
[b]Scientific founder

NAD(P) site in combination with several thousand specificity ligands [3]. The members of the bi-ligand libraries are small (<550 Da) and fulfill the drug-like criteria such as Lipinski's 'rule of five' [4]. Triad is also continuing to develop new CLMs for oxidoreductases and will expand these collections as it moves forward. Development has also started on bi-ligand inhibitors for protein kinases using the IOPE approach of pharmacofamily parsing, CLM discovery, NMR-directed chemistry and bi-ligand library construction. In addition, the technology platform has also been extended to include a single target structural approach termed NMR ACE. This approach shows Triad researchers how to link two-or-more small drug-like fragments into specific inhibitors. Although this approach does not currently have proteomic leverage, it does enable the researcher to attack individual targets within a gene family. This presents Triad and its partners with an opportunity to work on other target areas, including proteases, phosphatases and, in fact, any other proteins amenable to Triad's proprietary NMR spectroscopic analyses. Triad is currently in discussions with a number of potential partners about future collaborations. In the meantime, the company has been developing its first library (against oxidoreductases) and pursuing its own internal development efforts with the screening of several proteins against this new library.

Concluding remarks

While providing many new targets, recent advances in genomics and proteomics have been slow to deliver actual drug molecules. Triad's gene-family specific strategy for building libraries of probable drug molecules and the development of rapid screening of target proteins aims to deliver on the promise of proteomics. In addition, the extension of IOPE to address other targets using NMR ACE broadens potential targets beyond our current gene family approach.

Ultimately, the advantage of IOPE is that it enables Triad and its partners to generate lead compounds of high specificity and affinity in weeks, which is in stark contrast to the more traditional time frame of years. In addition, the technology platform is a way for clients to apply our proteomic leverage to their genomic information. This gene-family specific approach eliminates target-by-target searching and enables development of new therapies targeting gene families involved in multiple therapeutic areas.

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