A Work in Progress

What It Takes to Develop Your Targeted Therapeutic

LI-COR®

What is a targeted therapeutic?

Let's start with what a therapeutic is. A therapeutic is a substance used to treat a disease. An example of a therapeutic is chemotherapy for the treatment of cancer.

Targeted therapeutics are more specific. A targeted therapeutic is a substance that acts upon a particular target associated with the growth and spread of disease.

An example of a targeted therapeutic is bevacizumab, also known as Avastin. Avastin is used to treat several types of cancer, including lung and colorectal cancers. It works by blocking a protein associated with angiogenesis. Without this protein, the tumor cannot create new blood vessels. Avastin starves the tumor of resources, which prevents it from growing or spreading.

Why are scientists developing targeted therapeutics?

So, why are scientists developing targeted therapeutics? The short answer is–to improve human health outcomes from disease treatment.

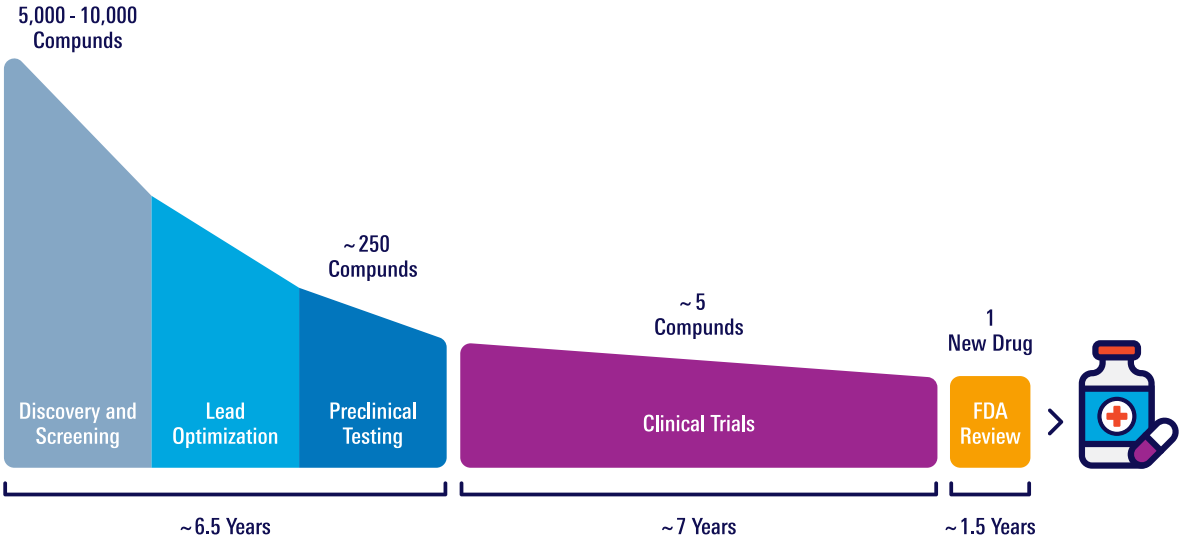
Chemotherapy is a powerful therapeutic used to treat many different cancers. However, because chemotherapy can damage cancer and healthy cells alike, it is notorious for side effects and a diminished quality of life for patients.

Because targeted therapeutics are intended to affect only their target cells, they offer several potential advantages, including:

- Less harm to normal cells
- Fewer side effects
- Improved effectiveness
- Overall improved quality of life

What does it take to develop a targeted therapeutic?

Developing a targeted therapeutic is a complex process that can take more than a decade. Over the development cycle, scientists identify, test, and eliminate thousands of targets and compounds. Compounds with therapeutic potential undergo hundreds of experiments and trials to ensure they are safe and effective–both in the short and the long term.



The process of discovering and developing a new therapeutic is filled with challenges, just a few of which are:

It is time-consuming

Bringing a new therapeutic to the market can take 10 to 15 years.

It can fail

At any point, a therapeutic can fail for multiple reasons, such as efficacy, toxicity, or not being the first to market.

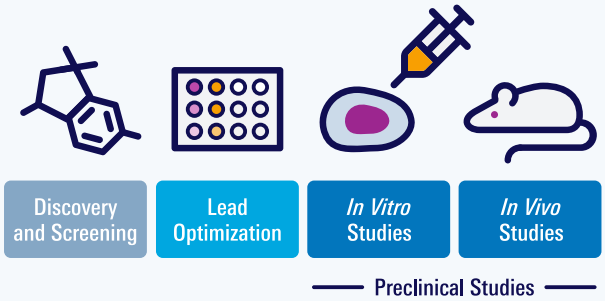
It is costly

It can cost upwards of several billion dollars to bring a new therapeutic to the market.

The Preclinical Research Stage

The first several years of targeted therapeutic development fall within the preclinical research stage. This stage includes many steps and experiments, all intended to identify and optimize candidates with the capacity to affect human health.

The most promising candidates from the preclinical research stage can then move into human clinical trials. From there, hopefully, a therapeutic will be approved and made available to the market.



What happens in the preclinical research stage?

There are many steps in this stage, each building on the other to identify and characterize a potential targeted therapeutic.

- Targets are identified and validated
- Compounds (or hits) are identified and validated
- Hits are optimized into leads
- Candidates are selected from the leads
- Candidates are characterized

Discovery

The preclinical research stage begins with discovery. During discovery, scientists identify targets. Targets are proteins seemingly involved in the initiation and progression of a disease. Scientists discover new targets by:

- Gaining new insight into a disease, such as learning more about its mechanism of action
- Developing new technology
- Screening various compounds
- Studying the effects of existing treatments

What are the qualities of a good target?

As scientists identify new targets, they are looking for those that:

- Have a known role in the disease
- Can be screened quickly
- Are not uniformly expressed
- Have a 3-D structure available to assess druggability
- Can be monitored for efficacy
- Have predictable side effects

Deciphering the Mechanism of Action of SALM3 via In-Cell Western™ Assay Using an Odyssey® CLx Imager

Example Study: "Structural basis of SALM3 dimerization and synaptic adhesion complex formation with PTPσ." Research conducted by Karki, S., et al., *Scientific Reports*.[1]

Scientists can gain new insight into a disease by studying its mechanism of action. For instance, synaptic adhesion-like molecules, such as SALM3, are vital for developing and maintaining neuronal connections. Dysfunction of these molecules is thought to contribute to the onset and progression of various neurological disorders. A better understanding of the structure of SALM3 and how it interacts with the signaling molecule PTPσ is critical for proper neuronal development and may guide future therapeutic development.

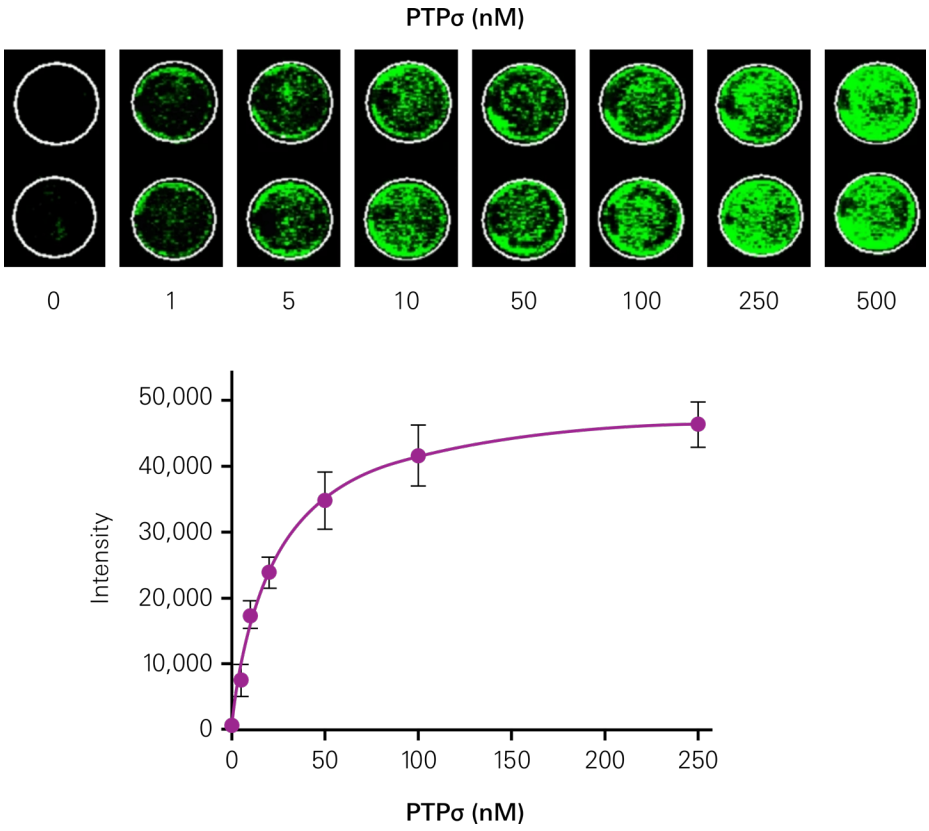


Figure 1. SALM3 demonstrates specificity when binding to PTPσ in HEK293T cells. HEK293T cells were transfected with SALM3-pDisplay plasmids and polyethylenimine. PTPσ-Fc was serially diluted (1-250 nM) in buffer. The transfected cells were then covered using 50 µl of diluted PTPσ-Fc and incubated overnight. Cells were detected using IRDye® 800CW Goat anti-Human Secondary Antibody. Images were acquired using an Odyssey CLx Imager. Adapted from Karki, S., et al. (CC BY).

Screening

After scientists have identified potential targets, they then validate the targets through screening. Screening determines which targets, out of many, produce a desirable effect. Validation demonstrates that manipulating the target can provide therapeutic benefits.

Scientists also screen compounds (a small molecule or biologic) to identify which ones can modify the behavior of the intended target. Compounds that affect the intended target are called "hits." A method commonly used by scientists to identify hits is high-throughput screening.

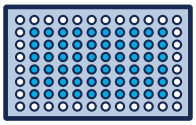
How do I screen my targets and compounds?

To screen your targets and compounds, you will need to develop assays that:

- Discriminate between potential targets
- Measure activity of potential therapeutics

You can quickly screen targets with excellent precision using the In-Cell Western™ Assay and an Odyssey® Imaging System.

1 Culture cells



2 Stimulate or inhibit cells

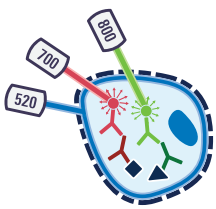
3 Fix and permeablilize cells

4 Incubate with blocking buffer

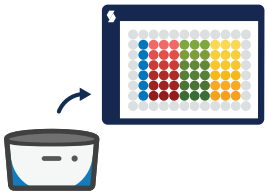
5 Incubate with primary antibody

6 Incubate with secondary antibody and CellTag™ 520 Stain

7 Excite with infrared lasers



8 Acquire image using Odyssey Imager



Confirming the Validity of an Assay to Detect 3CL^{pro} Inhibition Using the Odyssey® CLx Imager

Example Study: "Development of a Cell-Based Luciferase Complementation Assay for Identification of SARS-CoV-2 3CL^{pro} Inhibitors." Research conducted by Rawson, J.M.O., et al., *Viruses*.[2]

Screening compounds for therapeutic potential is critical. To develop antiviral therapeutics for COVID-19, researchers require a high-throughput cell-based assay to screen 3CL^{pro} inhibitors that can be performed in a biosafety level 2 setting. 3CL^{pro} is a protein essential for SARS-CoV-2 replication, which makes it an ideal target. However, current screening methods require a biosafety level 3 setting, so researchers developed a novel luciferase assay to identify 3CL^{pro} inhibitors within a biosafety level 2 setting.

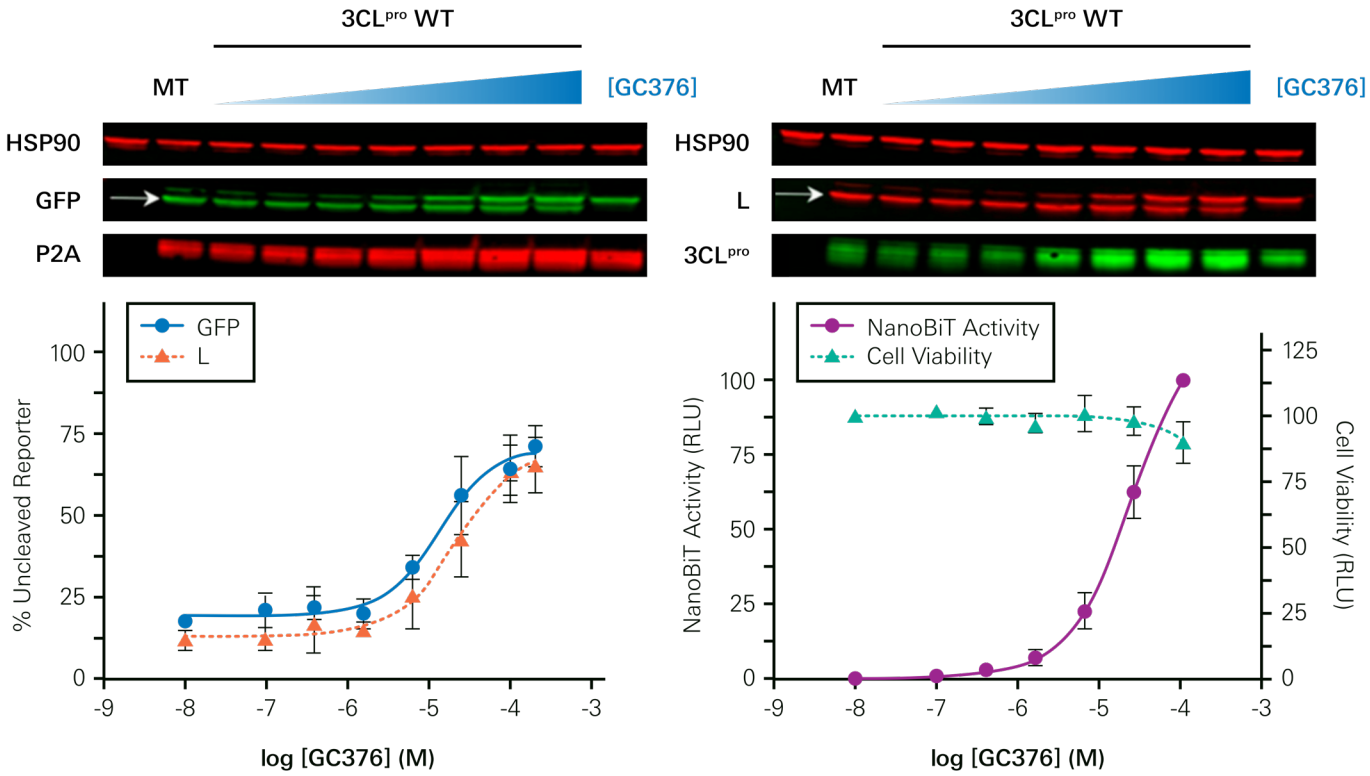


Figure 2. Western blot results confirm the ability of a novel luciferase assay to detect 3CL^{pro} inhibition. 293T cells were transfected using a lentiviral vector containing 3CL^{pro} and an S-L-GFP reporter. 4 hours post-transfection, DMSO or GC376 was added. After incubating for 30 hours, the cells were washed in PBS and lysed with a protease inhibitor. The blots were probed using rabbit anti-SARS-CoV 3CL^{pro}, mouse anti-P2A, mouse anti-L, or rabbit anti-GFP primary antibodies. Then the blots were incubated in IRDye® 680RD Goat anti-Mouse or IRDye 800CW Goat anti-Rabbit Secondary Antibodies. Signal was normalized to HSP90. Images were acquired using an Odyssey CLx Imager. With the Odyssey M Imager, one imager could perform the luminescence, cell viability, and Western blot assays. *Adapted from Rawson, J.M.O., et al. (CC BY).*

Lead Optimization

Scientists then confirm that their hits can modify their target. They also characterize hits for dose response, target affinity, and metabolic half-life. Leads are hits with desirable characteristics, such as high affinity, selectivity, and efficacy.

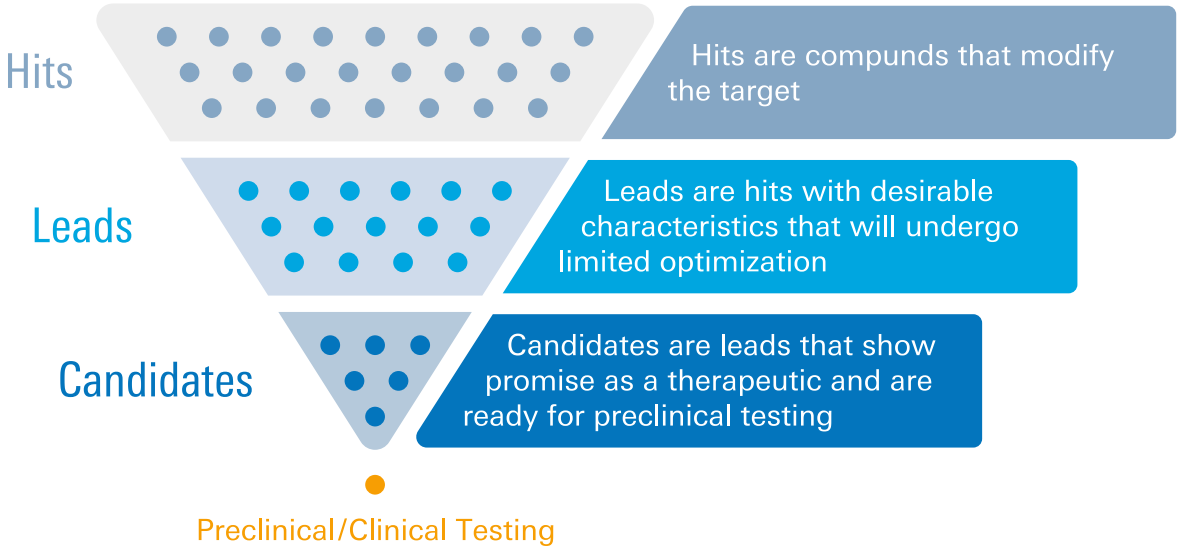
While leads are promising, they are not ready for preclinical testing. Instead, leads undergo limited optimization to:

- Enhance potency
- Increase target affinity
- Improve selectivity to reduce off-target effects
- Assess *in vivo* pharmacokinetics

High-quality leads are considered therapeutic candidates. Scientists study the candidates using *in vitro*, *in vivo*, and *ex vivo* assays to further evaluate which ones may enter clinical trials.

When developing a targeted therapeutic, the Odyssey® M Imager and Pearl® Trilogy Small Animal Imager are an ideal combination to cover your *in vitro*, *in vivo*, and *ex vivo* imaging needs.

What's the difference between hits, leads, and candidates?



Preclinical Testing

During preclinical testing, scientists perform *in vitro*, *in vivo*, and *ex vivo* assays to better understand a candidate's biological effects. They do this using both cellular and animal disease models. Candidates cannot move onto human clinical trials until scientists have characterized markers of efficacy and safety, including:

- Biological processes
- Affinity and selectivity
- Toxicity and adverse effects
- Drug-drug interactions

Preclinical testing confirms the targeted therapeutic is effective and is unlikely to cause serious harm. It also helps scientists dial in the delivery method and dosage.

Assessing Specificity and Colocalization of 800CW-TATE *ex vivo* Using an Odyssey® CLx Imager

Example Study: "Necrosis binding of Ac-Lys^0(IRDye800CW)-Tyr^3-octreotate: a consequence from cyanine-labeling of small molecules." Research conducted by Stroet, M.C.M., et al., *EJNMMI Research*.[3]

It's important that scientists understand the biological effects of a candidate. For example, tumors contain and are surrounded by necrotic tissue. Cyanine-labeled peptides can bind to both dead and living cells. Scientists should be aware of this behavior when developing targeted probes. It can make the tumor and surrounding diseased tissue easier to detect for image-guided resection but may present issues with drawing conclusions about target presence based on signal intensity.

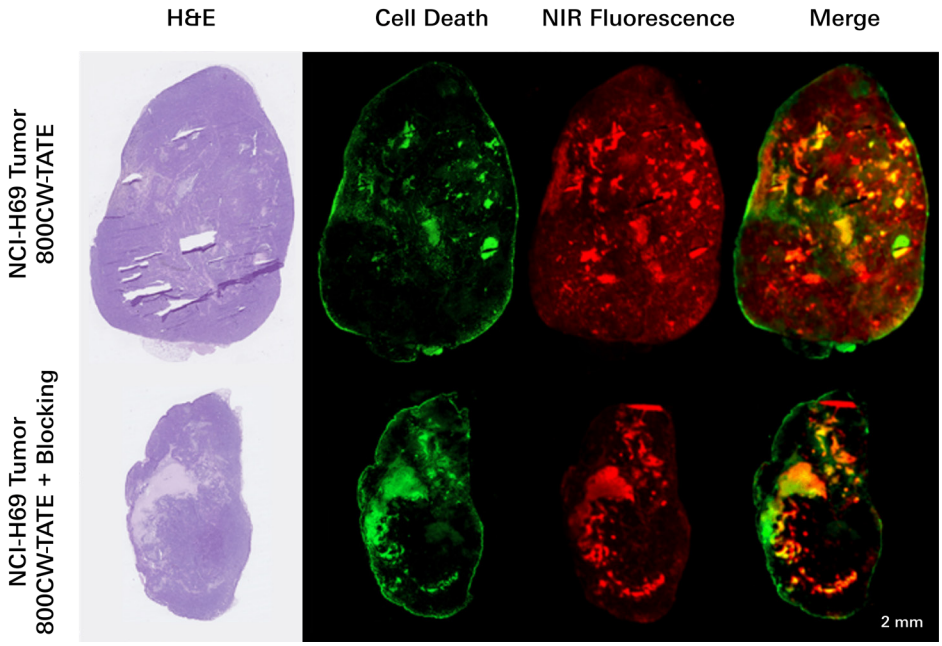


Figure 3. 800CW-TATE demonstrates binding specificity to $SSTR_2$ in living and dead cells. A mouse xenograft bearing a human small cell lung cancer (NCI-H69) tumor was injected using 3 µg 800CW-TATE (top). A similar xenograft was first injected with 3 mg DOTA-TATE to block $SSTR_2$ binding and was later injected with 3 µg 800CW-TATE (bottom). The tumors were then removed, sliced into serial sections, and stained. NIR fluorescence images were acquired using Odyssey CLx Imager. Adjacent sections were also imaged on microscopes using TUNEL to detect cell death and H&E stain. With the Odyssey M Imager, similar images could be acquired at the same resolution and at the same time. *Adapted from Stroet, M.C.M., et al. (CC BY).*

What can I learn from *in vitro* studies?

Studies performed in vitro use a cellular disease model with a pathology nearly identical to that seen in human disease. Studying disease models using *in vitro* assays, such as the In-Cell Western™ Assay, enable you to:

- Characterize and validate selectivity and affinity
- Understand disease progression
- Test treatment approaches
- Identify candidates to be refined or eliminated

What can I learn from *in vivo* studies?

Candidates are evaluated by studying animal disease models *in vivo*. These studies reveal candidate activity in live animals and provide information about characteristics that you cannot see *in vitro*. Ultimately, *in vivo* studies should answer the following questions about a candidate.

- How is it absorbed, distributed, metabolized, and excreted?
- What are the potential benefits and mechanisms of action?
- What side effects or adverse events are there?
- What is the optimal dosage and delivery method?
- How does it interact with other drugs?

What can I learn from *ex vivo* studies?

A continuation of *in vivo* studies, *ex vivo* studies enable you to further characterize your therapeutic candidate. These studies look closely at where your candidate accumulates in excised tissue and organs. Examining excised tissue and organs allows you to better assess:

- Uptake
- Distribution and localization
- Clearance
- Toxicity

Observing Colocalization of CD4 T Cells and Denatured Collagen *in vivo* Using a Pearl® Imager

Example Study: "Lack of the MHC class II chaperone H2-O causes susceptibility to autoimmune diseases." Research conducted by Welsh, R.A., et al., *PLoS Biology*.[4]

Scientists can use *in vivo* studies to visualize disease states or candidate distribution throughout the body. Millions are affected by autoimmune disease. A better understanding of the contributing risk factors may help in early identification and intervention for improved outcomes. This study examines an apparent link between DO, an accessory molecule found in the Major Histocompatibility Complex class II pathway, and susceptibility to various autoimmune diseases.

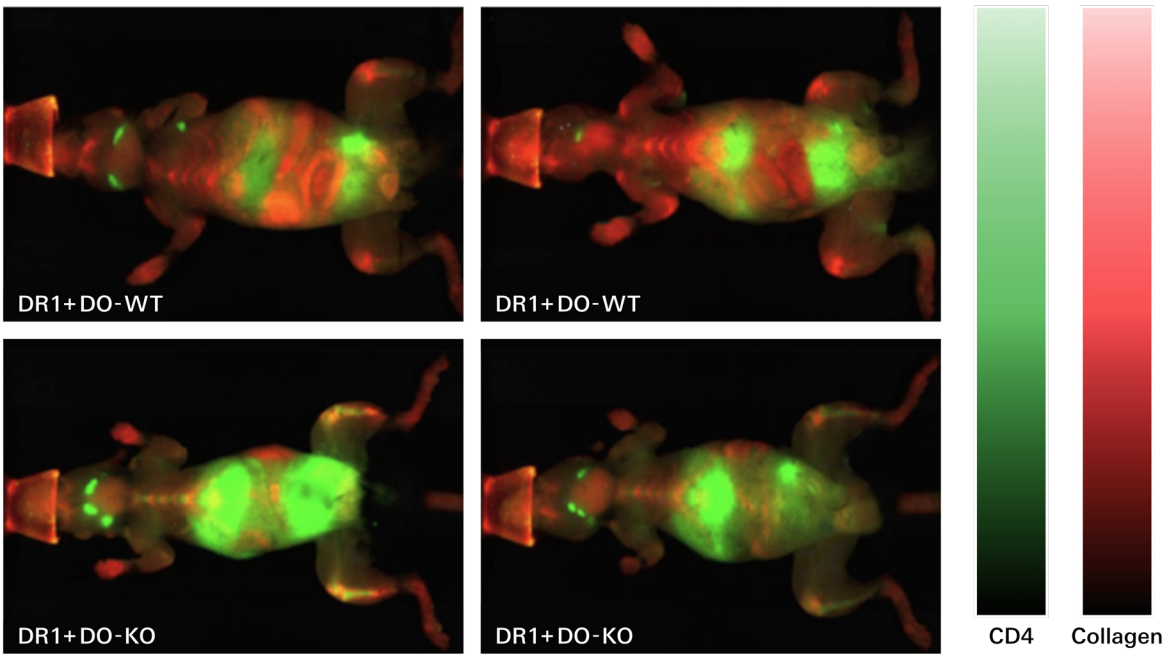


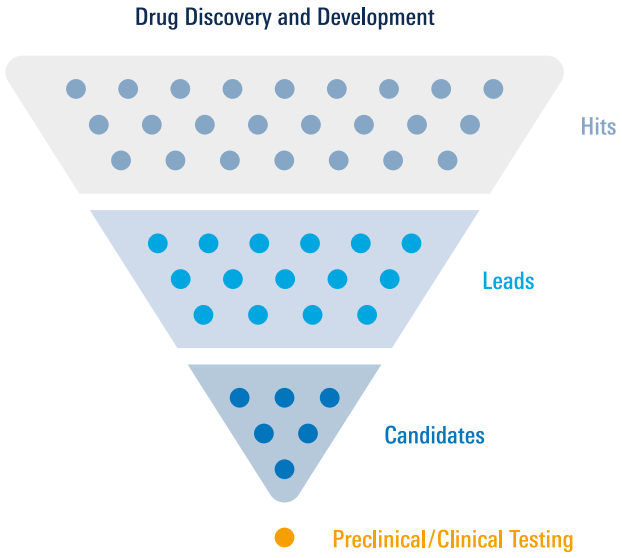
Figure 4. Colocalization of CD4 T cells and denatured collagen was observed only in the diseased joints of DR1+DO-KO mice. 48 hours before imaging, DR1+DO-KO and DR1+DO-WT mice with collagen-induced arthritis were injected intravenously with a collagen mimetic peptide probe labeled with IRDye® 680RD (red) and intraperitoneally with a CD4 probe labeled with IRDye 800CW (green). Colocalization (orange) of CD4 T cells and denatured collagen was found only in affected joints of DR1+DO-KO mice, suggesting increased susceptibility to rheumatoid arthritis in these mice. Images were acquired using a Pearl Imager. *Adapted from Welsh, R.A., et al. (CC BY).*

What's Next

From a pool of thousands of possible targets and compounds, scientists will have reduced the number of candidates to just a few. These are candidates that, through rigorous scientific effort and testing, have shown significant therapeutic potential with minimal toxicity. The candidates will undergo several phases of human clinical trials before moving on to secure FDA approval.

Just getting a targeted therapeutic to clinical trials is an enormously challenging, time-consuming, and costly endeavor. Additionally, there is no guarantee that the targeted therapeutic won't fail for one reason or another–even at this point.

LI-COR offers the latest in imaging technology, reagents, software, and expertise to enable you to make decisions about your targeted therapeutic quickly and with confidence.



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02/22 997-19950