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Genetic Engineering & Biotechnology News
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Scotland Steadily Builds a Biotech Presence

Homeland of Dolly the sheep continues to step up its game in the biomedical field. **p. 10**



Classic Medicine Awaits Digital Overhaul

E-records could change the structure of clinical trials and scope of personalized medicine. **p. 46**

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microRNAs are getting closer to realizing potential as biomarkers and therapeutic targets. **p. 32**

> HCV Treatment Enters Transformative Era

Thanks to new antiviral therapies, chronic hepatitis C could soon be curable in most patients. **p. 53**

Nudging Difficult-to-Express Proteins

Emerging Solutions Aim to Bring Challenging Targets Under Control

Josh P. Roberts

Some proteins are easier to express than others. For some, just clone them into an E. coli vector and let the bacteria do the work. But for others—such as toxic proteins, membrane proteins, glycosylated proteins, and hydrophobic proteins—getting them to express, fold, and function or crystallize can present a challenge.

Scientists at CHI's "PEGS" conference, held last month in Boston, were keen to talk about some of the difficulties they faced in expressing challenging proteins. Fortunately, they were just as keen to talk about the solutions they came up with.

Typically, when researchers want to express a protein they turn first to E. coli: transfect the bacteria with the appropriate vector, grow it up, plate it out, select colonies, and grow up cultures of



According to Thermo Fisher Scientific scientists, there are advantages to expressing human proteins using a human system, foremost among them are that the products will be properly folded and properly post-translationally modified.

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Small Molecule Simplicity Gives Them Staying Power

Chemical Entities Boast Some Distinct Advantages over Biological Entities

K. John Morrow Jr., Ph.D.



Areva Pharmaceuticals is focused on discovering, developing, and commercializing drugs that target GPCRs in four major therapeutic areas: cardiovascular, central nervous system, inflammatory, and metabolic diseases.

There is a profound association between cancer and inflammatory processes, according to Raffaella Sordella, Ph.D., assistant professor at Cold Spring Harbor Laboratory. Dr. Sordella was a keynote speaker at CHI's recent conference on drug discovery chemistry.

Discussions at the meeting about small molecule approaches to anti-inflammatory therapies devoted considerable time to the link between malignancy and inflammation. Chronic inflammation not only predisposes individuals to cancer and correlates negatively with cancer prognosis, but inflammation may, in fact, hamper tumor response to drug treatment.

The Janus kinases, or JAKs, a family of intracellular, nonreceptor tyrosine kinases that transduce cytokine-mediated signals, are an important target for drugs directed against cancer and inflammatory processes. Incyte (www.incyte.com) has developed a number of candidate compounds including INCB18424, according to James D. Rodgers, Ph.D., executive director of medicinal chemistry.

"Our rationale is based upon the observation that dysregulation of the JAK-STAT pathway is a hallmark of both chronic inflammatory diseases and myeloproliferative neoplasms," Dr. Rodgers explained. "Indeed, a single

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Sticky ends

▶ **Syngenta** plans to construct \$71 million biotech research facility next to existing campus in Research Triangle Park to focus on discovering and developing new agronomic traits... ▶ **Watson Pharmaceuticals** acquired **Specifar Pharmaceuticals**... ▶ **Smith & Nephew Orthopedics** signed deal with **Graftys** to obtain distribution rights for Graftys' macroporous calcium phosphate bone void filler... ▶ **Labtec** opened GMP manufacturing facility for transdermal patches and oral films just south of Hamburg, Germany... ▶ **PsychoGenics** entered into strategic alliance with **Evotec** to provide integrated CNS drug discovery solutions to pharmaceutical and biotech firms... ▶ **Sigma-Aldrich** acquired all outstanding shares of chemical supplier **Vetec Quimica Fina** to strengthen its position in Latin America... ▶ **Bruker** opened new center of excellence at the Biopolis, Singapore's biomedical hub... ▶ Officials at **Biologics Process Development**, a wholly owned subsidiary of **Vipropro**, say they will be doubling the size of the firm's existing bioprocess and scale-up laboratory in Poway, CA.

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amino acid mutation, V617F, in the JAK2 gene was recently identified in a variety of such conditions including polycythemia vera, essential thrombocythemia, and myelofibrosis."

The mutant JAK2 leads to unchecked signaling through cytokine and growth factor receptors without the need for the ligands. This permits phosphorylation and activation of STATs (signal transducers and activators of transcription proteins). The activated STATs dissociate from the receptor and form dimers, then migrate to the cell nucleus where they regulate transcription of selected genes.

It is well known that transgenic mice that do not express JAK1 have defective responses to some cytokines such as IL-6 and interferon-gamma (IFN- γ). Although there are four members in the JAK family of kinases, the company focused on developing selective

JAK1 and JAK2 inhibitors based on its understanding that IL-6, a clinically validated target in autoimmune diseases such as rheumatoid arthritis, signals through JAK1 and JAK2.

An additional piece of the puzzle is the observation that IL-6 is also involved in the pathogenesis of myeloproliferative neoplasms. With knowledge of JAK inhibitors originally developed by Merck and Pfizer, Dr. Rodgers and his colleagues have investigated a number of polycyclic molecules with respect to their inhibition spectrum, oral bioavailability, and pharmacokinetics.

"We designed a series of potent and selective JAK1 and JAK2 inhibitors which we studied in an IL-6 driven cell line," Dr. Rodgers continued. After many rounds of discovery, evaluation, and elimination, the company selected the compound INCB018424.

"We completed Phase III trials for the treatment of myelofibrosis, and we are on schedule to file a new drug application in the second quarter of 2011."

The JAK pathway is also a validated therapeutic target for psoriasis and potentially other inflammatory cutaneous diseases.

Targeting IL-17 Release

Daniel Vitt, Ph.D., is CSO for R&D at 4SC (www.4sc.com), where he directs studies on IL-17, one of the key cytokines responsible for autoimmune dysfunction. The company has introduced vidofludimus, an orally available dual inhibitor of IL-17 A and F as well as dihydro-orotate dehydrogenase (DHODH), used in the treatment of rheumatoid arthritis and inflammatory bowel disease.

According to Dr. Vitt, the company is

currently pursuing an inhibitor-optimization program including a family of molecules with sub nanomolar inhibitor activity. The goal of the program is to build a new systemic baseline therapy for the treatment of IL-17 associated autoimmune diseases including psoriasis, multiple sclerosis, and lupus erythematosus.

IL-17 is produced by subset of T cells known as T helper 17 cells (T_H17), which play a role in autoimmune diseases. Dr. Vitt noted that they are upregulated in psoriatic tissue, and thus, the inhibition of IL-17 and IFN γ hold promise as a treatment for psoriasis. IL-17A, the founding member of the IL-17 family, is one of the key cytokines for driving and maintaining rheumatoid arthritis.

In clinical trials, Vidofludimus showed an 89% positive response rate in the treatment of inflammatory bowel disease, indicating a broad potential in ameliorating immune dysfunction.

In vitro studies demonstrate that it is active specifically against T-cell cytokines, inhibiting T and B cell proliferation and IL-17 A and F release. The suppression of the immune response is apparently through the inhibition of DHODH, an enzyme required for the synthesis of DNA and RNA. It is noteworthy that, while cell proliferation reestablishes itself after addition of uridine, IL-17 release appears to be permanently shut off.

Dr. Vitt and his colleagues are now investigating next-generation compounds with markedly improved activity over vidofludimus. One of these, SC89732, has been shown to shut down IL-17 A and F production as well as IFN- γ at low nanomolar concentrations, while having no effect on IL-1 β in activated T cells.

A second candidate compound, SC92366, has been evaluated for in vitro cellular toxicity and specificity. It showed no cytotoxic effect on unstimulated peripheral B lymphocytes, nor any kinase-inhibiting effects.

Macrolides as Anti-Inflammatories

Cempra Pharmaceuticals (www.cempra.com) is investigating the anti-inflammatory properties of macrolide antibiotics conventionally employed as antibacterial agents. Prabhavathi Fernandes, Ph.D., CEO, discussed the search for macrolides with low activity as antibiotics and elevated performance in treating chronic inflammatory disease.

"The antibacterial effects can be differentiated from the anti-inflammatory effects, especially in late-stage chronic inflammatory disease. We have found that the erythromycin molecule is desirable as a chemical backbone because it concentrates in tissues including the lung, macrophages, and dermis."

However, it has not been approved for long-term treatments due to its propensity to select for resistant bacterial strains. For this reason, the company is searching for alternative structures that display lower antibiotic activity and elevated anti-inflammatory properties.

The current model forming the theoretical framework for these investigations is based on oxidative stress triggering gene repression through the activation of PI3K, or phosphatidylinositol 3' kinase, an enzyme known to

play a critical role in tumorigenesis. When PI3K phosphorylates the histone deacetylase 2 (HDAC2), the enzyme is destroyed and an inflammation cascade takes place.

Based on these considerations, molecules that block PI3K should possess anti-inflammatory and antitumor activities. And indeed, in animal and in vitro models, the macrolides erythromycin, clarithromycin,

and solithromycin demonstrated anti-inflammatory effects.

Currently, Cempra is pursuing two modified ketolide molecules, A3 and A6. "Our lead macrolides have little antibacterial activity and belong to a series with little CYP3A4 (a mixed function oxidase), hERG (a potassium ion channel), or motilin (housekeeper of the gut) inhibition."

John Robinson, Ph.D., a research investigator at Array BioPharma (www.arraybiopharma.com), discussed the PIM kinase family, consisting of three serine/threonine kinases. The PIM genes are induced by STAT and function as a critical signaling node downstream of cytokine, growth factor, and oncogene pathways, thereby regulating a va-

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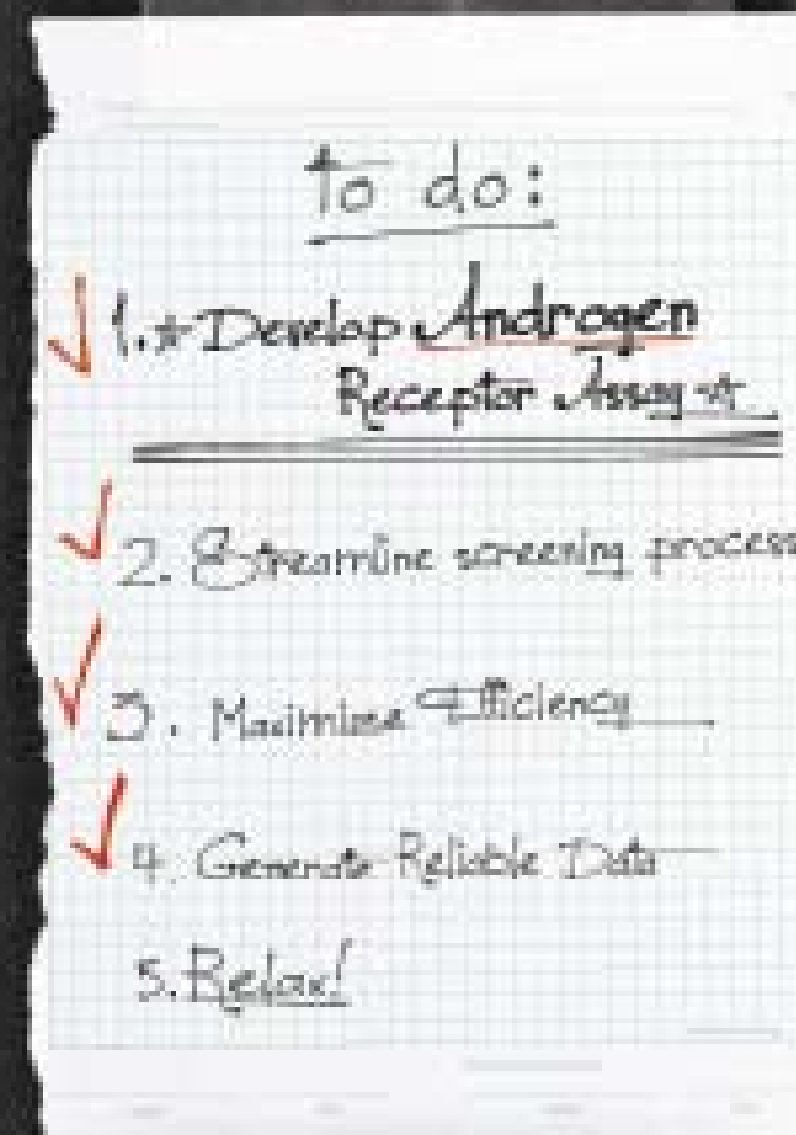
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riety of cellular functions.

"We originally picked PIM as a possible entryway into antitumor agents because of its relationship to STAT, and the fact that many oncogenes are known to drive through STAT-3/5," Dr. Robinson said.

Because of their pivotal regulatory function, Array BioPharma spent six to eight months creating a number of selective inhibitors with

high solubility and permeability as well as oral bioavailability. One of these, AR460770, is a potent and selective PIM 1/3 inhibitor.

Targeting PIM Kinases

The compounds were evaluated for their antitumor effects, but these were found to be modest. Since PIM is not a primary oncogene, Array investigators turned their at-

tention to the anti-inflammatory potential of the compounds.

The Array team then focused on T cells, and found that their panel of compounds exerted an antiproliferative effect. They further observed that in T cells, compounds that inhibit PIM 1/3 cause a decrease in T_H17 mediated cytokine production, as well as inhibit proliferation of CD4+ T cells.

Moreover, in a mouse experimental autoimmune encephalomyelitis model, AR460770 successfully inhibits ataxia and subdues relapses; only 1 out of 15 animals suffered relapse when treated continuously. "In certain settings, PIM 1/3 inhibitors differentiate from JAK inhibitors, but the clinical consequences of these observations will have to be worked out in patient trials," Dr. Robinson stated.

When evaluated in a mouse lupus model, the same compound negated proteinuria, which is a hallmark of the condition, and showed a marked reduction in kidney damage when histopathology studies were evaluated. In a similar vein, in a mouse inflammatory bowel disease model, treatment with the PIM inhibitor AR472317 showed efficacy equivalent to the anti-IL-12p40 antibody.

"While there appears to be a relationship between the oncological and autoimmune effects of the PIM system, we don't know exactly the key that ties them together, and this will have to be worked out through future investigations."

S1P₁R Agonists

Robert M. Jones, Ph.D., senior director of medicinal chemistry at Arena Pharmaceuticals (www.arenapharm.com), and his colleagues are investigating agonists of the sphingosine-1-phosphate receptor (S1P₁R) as a treatment for multiple sclerosis.

Multiple sclerosis is characterized by the activation of autoimmune lymphocytes in the periphery; they mature and proliferate in the lymphatic system and then pass into the blood and cross the blood brain barrier where they cause inflammation, demyelination, and axonal damage. For decades the etiology of this condition has been in doubt.

The S1P₁R agonists activate and internalize the receptor, thereby blocking the sphingosine phosphate-dependent migration of activated T cells from the lymph nodes to efferent organs. This mechanism results in a decline in the level of T cells in the brain and leads to a decrease in the levels of cytokine markers such as IL-17 and IL-22, as well as a dampening of the inflammation within the brain.

Dr. Jones and his colleagues have generated a series of modified molecules, starting with in-house screening hits. Moving through several iterations, they eventually arrived at the compound APD334.

This molecule has been evaluated against a mouse model of experimental autoimmune encephalitis. It showed a powerful prophylactic effect in preventing the disease in animals receiving increasing doses of the drug, and was also effective in the therapeutic mode after the disease had been induced. Moreover it prevented the infiltration of T lymphocytes into the brain, suggesting it may be useful for the treatment of MS.

In recent years, antibodies have been the area of pharmaceutical research that has generated the most excitement. Though small molecule agents have flourished in recent years, they have retained significant advantages over biological entities in terms of storage, delivery, and specificity. **GEN**

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