Novel TACE Inhibitor

by Allosterix Ltd.
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Executive Summary

Tumor necrosis factor-alpha (TNF-α) is a multifunctional protein that plays a key role in fighting infection, eradicating tumors, and the inflammatory response. TNF-α is a potent pro-inflammatory cytokine that is released to perpetuate the inflammatory signal throughout the body. Excessive production of soluble TNF-α has been implicated as the primary mediator for several chronic, immune-based pathologies such as rheumatoid arthritis, Crohn's disease, and plays a central role during acute inflammatory episodes, such as ischemic stroke and endotoxic shock (Erzurum, 2006).

Over the past decade, the important contributory role that TNF-α plays in immune-based disorders has led to the discovery of a number of agents which antagonize the effects of circulating TNF-α. These agents, termed "biologics" because they are derived from living organism, have become a vital component of the rheumatologist's armororium against inflammation-base disorders.

Although substantial progress has been made with the use of biologic agents, they are reserved for patients that do not respond to first line treatment. This is due to the prohibitive cost of manufacturing and administering the drug, the side effects associated with intravenous administration, and the possibility of inducing allergic response to the drug itself (Finckh et al., 2006). Moreover, some patients still have poorly or incompletely controlled disease with these therapies (Olsen and Stein, 2004), with non-responsive rates ranged from 20% to 50%, depending on disease stage (Feldmann and Steinman, 2005; de Vries and Tak, 2005).

Therefore, the discovery of new compounds that can be administered orally, with a similar mechanism of action to that of the biological agents, would significantly impact patient outcome in terms of symptom resolution and disease progression.

The TNF-α converting enzyme (TACE) has emerged as the main TNF-α cleaving enzyme and has been considered a principal therapeutic target for the treatment of TNF-dependent pathologies. For over a decade, several big pharmaceutical companies have been actively developing and testing TACE inhibitors with drug-like properties. Clinical trials with TACE inhibitors confirm that TACE inhibition can effectively modulate TNF-α levels and decrease painful swelling associated with arthritis. These experiments validate the hypothesis that TACE inhibition is an effective means of treating the underlying cause of chronic inflammatory conditions by regulating TNF-α release. However, many of the TACE inhibition programs have been discontinued. The reasons for department closure varied, where one company sighted a lack of efficacy and while another company had concerns regarding liver toxicity. Allosterix Pharma has developed a series of potent, exclusively selective, and orally available TACE inhibitors, which can meet the current demand for better therapies. Our candidate peptides are able to inhibit TACE in the pico to nanomolar range. They reduced TNF-α levels in murine cells from 10 to 55%. After testing our most potent lead compounds for stability in human serum assay, two lead compounds have qualified for animal experiments. They were both tested successfully for toxic effects in mice and one is currently being tested at concentration range of 0.05-25 mg/kg in collagen-induced arthritis mice model.
The Current Market

Inflammation and Tumor Necrosis Factor-α

Inflammation is a complex biological response to harmful stimuli, such as pathogens or irritants. It is a protective mechanism to remove offending agents and initiate the healing process. In the absence of inflammation, wounds and infections would not heal and progressive destruction of the tissue would compromise the organism's survival. However, inflammation that runs unchecked can lead to a host of acute and chronic immune based disorders.

Tumor necrosis factor-alpha (TNF-α) is a multifunctional protein and plays a key role in fighting infection, eradicating tumors, and mediating the acute and chronic inflammatory effects of the immune system (Black et al., 1997). When immune cells become activated, TNF-α is cleaved and released into circulation to propagate the inflammatory signal throughout the body. Excessive production of TNF-α has been directly implicated in a wide variety of diseases that differ considerably in their etiology and clinical manifestations. These include rheumatoid arthritis, psoriasis, Crohn’s disease, ankylosing spondylitis, and refractory asthma (Erzurum, 2006).

Therapies Targeting Immunologic Mediators

Over the past decade, the important contributory role that TNF-α plays in immune-based disorders has led to the discovery of a number of agents which antagonize the effects of circulating TNF-α either via direct interaction with the molecule or its receptor. The most notable anti-TNF-α agents that have received FDA approval as effective therapies for RA, psoriasis, Crohn’s disease, and ankylosing spondylitis are: etanercept (Enbrel®; Amgen), infliximab (Remicade®; Centocor), and adalimumab (Humira®; Abbott Laboratories) (Olsen and Stein, 2004). Since the advent of TNF-α biologics, several other immunogenic targets have been successfully targeted with this approach, such as the T-cell directed leflunomide (Arava®: Sanofi) and abatacept (Orencia®: Bristol Myers Squibb).

Although substantial progress has been made with the use of these biologic agents, they are reserved for patients that do not respond to first line treatment. This is due to the prohibitive cost of manufacturing and administering the drug, the increased risk of serious infection, the side effects associated with intravenous administration, and the possibility of inducing allergic response to the drug itself (Finckh et al., 2006).

TACE and the Release of TNF-α

There has been a collective effort to develop alternate therapeutic strategies to control soluble TNF-α levels. Human TNF-α is bound to the surface of several cell types, however once the immune cells encounter a foreign agent, they become activated and trigger a cascade of biochemical reactions. During this initial response, an enzyme will cleave TNF-α to release the soluble form into circulation, and TNF-α can enter the circulation to propagate the inflammatory signal throughout the body. The main enzyme responsible for cleaving TNF-α is a membrane-associated metalloprotease (MMP) named the TNF-α converting enzyme or TACE (McGeehan et al., 1994; Black et al., 1997).
Previously tested TACE inhibitors successfully decreased the quantity of circulating TNF-α and proved to be an effective means of regulating soluble TNF-α levels (Ott et al., 2008). In one experiment, cells deficient in TACE released only 10% of the normal amount of TNF-α when stimulated with a powerful inflammatory trigger (Black et al., 1997). In preclinical studies, Bristol Myer’s Squibb found their TACE inhibitor to be as effective as anti-TNF-α agents at reducing inflammation score (Qian et al., 2007). These experiments validate our hypothesis; TACE inhibition can effectively modulate perpetual TNF-α signaling and this is an effective means of treating the underlying cause of chronic inflammatory conditions.

**TACE Inhibitor: Development and Pipeline**

Researchers have actively pursued TACE as pharmacological target because of the central role it plays in releasing TNF-α and because TACE inhibitors would be amenable to the production of orally available drugs. An overview of the progress made from preclinical to clinical studies for TACE is summarized in Table 1. Despite the success in clinical trials, there are no TACE inhibitors on the market. We feel that our approach to TACE drug design will revolutionize this area of research and provide a novel, much needed, therapy for TNF-α-based disorders.

Dual MMP–TACE inhibitors GW3333 and GW4459 from GlaxoSmithKline (GSK), PKF 242 and PKF 484 from Novartis, and TMI-001 from Wyeth proved to be efficacious in various animal models ranging from arthritis induced by collagen (collagen-induced arthritis (CIA)) to the more severe adjuvant-induced arthritis (Table 1) (Moss et al., 2008). However preclinical studies were discontinued due to the development of liver toxicity.

Extensive efforts have been made to modify broad range MMP inhibitors (TMI-001) into selective compounds with drug-like properties. BMS-561392 by Bristol-Myers Squibb (BMS) and TMI-002 from Wyeth, showed increased affinity than their predecessors and were able to decrease inflammation in the mouse CIA model. These results are important as they provide preclinical validation that TACE inhibition is beneficial for disorders associated with excessive TNF-α. In fact, a 10.5 mg/kg dose of BMS-561392 twice daily worked as well as Enbrel® at reducing inflammation and a much lower dose, 2.8 mg/kg day, was more effective than Remicade® (Grootveld and McDermott 2003).

Two TACE inhibitors reached phase II clinical trials but were not pursued due to lack of efficacy (TMI-005) and liver toxicity (BMS-561392) (Thabet MM & TW Huizinga 2006; Car 2007). Both of these inhibitors showed some inhibitory activity against other MMPs and it remains unclear whether selective inhibition of TACE would illicit liver toxicity. Compounds GW3333 and TMI-005 would have been considered for other indications; however the development of tendonitis prevents their advancement beyond Phase II clinical trials.

Despite the success of BMS at developing potent TACE inhibitors, their program has been discontinued over of concerns of mechanism-based liver toxicity. It remains unclear whether hepatotoxicity is the direct result of TACE inhibition or that of non-selective MMP inhibition. However, Wyeth and Bayer Schering are still actively pursuing TACE inhibitors and both companies have filed patents with broad claims of action in the past two years.
<table>
<thead>
<tr>
<th>Company</th>
<th>Drug</th>
<th>Target</th>
<th>Preclinical</th>
<th>Clinical</th>
<th>Status</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wyeth</td>
<td>TMI-005 (apratasiat)</td>
<td>TACE MMP RA</td>
<td>EC_{50} 87nM in CIA</td>
<td>PII completed: no incr ACR20 thus ineffective. Perhaps due to basal TNF levels or memTNF activity. No liver toxicity but tendonitis was reported</td>
<td>Discontinued March 2007</td>
<td>Thabet &amp; Huizinga 2006 Moss et al, 2008 CT.gov database</td>
</tr>
<tr>
<td>Wyeth</td>
<td>New formulation</td>
<td>TACE A/IBD</td>
<td>IC_{50} 1.5nM to TACE</td>
<td>PII</td>
<td>Active since Sept 2006</td>
<td>232nd ACS (2006)</td>
</tr>
<tr>
<td>Wyeth</td>
<td>Tpl2 inhibitor</td>
<td>TNF-α cleaving enzyme</td>
<td></td>
<td></td>
<td>Still Active</td>
<td>232nd ACS (2006)</td>
</tr>
<tr>
<td>BMS</td>
<td>BMS-561392</td>
<td>Partially selective TACE RA IBD</td>
<td>IC_{50} 0.14 nM Bioavail 35% Potent LPS-induced TNF suppressor. Mech. liver toxicity</td>
<td>PII: CIA good results Toxicity in liver Hypothesize due to TACEs other targets and R-sensitivity.</td>
<td>Discontinued Sept 2004</td>
<td>Moss et al, 2008 CT.gov database</td>
</tr>
<tr>
<td>BMS</td>
<td>DPC-A38088 * Modified BMS-561392</td>
<td>TACE specific RA</td>
<td></td>
<td></td>
<td>Discontinued</td>
<td>Moss et al, 2008 CT.gov database</td>
</tr>
<tr>
<td>BMS</td>
<td>BMS-566394</td>
<td>Selective TACE RA</td>
<td>Liver damage in rats</td>
<td>Found articles related to formulation</td>
<td>Discontinued</td>
<td>Car 2007</td>
</tr>
<tr>
<td>BMS</td>
<td>DPC333</td>
<td>Selective TACE RA</td>
<td>Rats, dogs, chimps LPS/CIA IC_{50} 113nM</td>
<td>PII completed to establish pharma- kinetics</td>
<td>Status Unknown</td>
<td>Grootveld &amp; McDermott 2003</td>
</tr>
<tr>
<td>GSK</td>
<td>GW4459</td>
<td>MMP TACE RA</td>
<td>IC_{50} 4.3 nM</td>
<td></td>
<td>Discontinued 2002</td>
<td>Moss et al, 2008</td>
</tr>
<tr>
<td>GSK</td>
<td>GW3333</td>
<td>MMP TACE</td>
<td>IC_{50} 42nM and 19uM for T, M. Effective in animals CIA Caused tendonitis</td>
<td></td>
<td>Discontinued 2001</td>
<td>Moss et al, 2008</td>
</tr>
<tr>
<td>Novartis</td>
<td>PKF 242 PKF 484</td>
<td>MMP/ TACE RA</td>
<td></td>
<td>PII halted</td>
<td>Discontinued March 2004</td>
<td>CT.gov database</td>
</tr>
<tr>
<td>Roche</td>
<td>R-618</td>
<td>RA IBD</td>
<td>PII</td>
<td>Status Unknown</td>
<td></td>
<td>CT.gov database</td>
</tr>
</tbody>
</table>

**Table 1.** Overview of preclinical and clinical results for TACE inhibitors in the last 10 years
Allosterix Drug Design Model

All of the TACE inhibitors developed to date have been orthosteric in nature, meaning they target the active of the enzyme. Almost always, these active sites are conserved among families of enzymes and much time and effort is expended to create drugs with a selectivity profile fit for human use. In contrast, allosteric sites are located distal to the active site, in non-conserved regions of the enzyme. Therefore, by designing drug to these unique regions we can produce inhibitors with unprecedented selectivity and dramatically decrease the likelihood of triggering unwanted side effects. This is a departure from any previous drug design strategy aimed at inhibiting enzymatic function.

Allosterix specializes in the novel drug design of inhibitors based on the principles of allostery. We have developed patent-pending technology for locating allosteric sites and peptidic drug design. The advantage to this drug-design approach is simple: specificity. With our approach, selectivity is inherent in each peptidic inhibitor.

Allosterix's TACE Inhibitors

Using Allosterix technology, we have designed and tested 32 drug-like compounds. From our in vitro screening assay, we identified three water soluble drug candidates that inhibit TACE in the pico to nano molar range and are slow binders (equilibrium was not achieved after ~24hrs). Our best lead-candidate AL4_1A1 (D-peptide) had IC\textsubscript{50}=99±21 fM (figure 1.).
The three lead molecules were tested for stability in human serum.
To avoid activity assay disruption by the serum, inhibitors AL24-2A1, AL4-1A1 and AL-Rev1, were incubated in buffer containing 10% serum for four hours and then diluted only in buffer to the wanted concentration of the inhibitor. Thus the final serum concentration reached 0.275% and did not harm the activity assay. The inhibitor and enzyme were incubated for 4 or 18 hours and tested in activity assay. As before data extracted is $V_{\text{int}}$ and $R_{\text{sqn}}$ for each inhibitor concentration.

After experiments with this lead molecules were revealed certain decrease in inhibition activity. For AL24-2A1 inhibition loss was about 80%, for AL-AL-Rev1 about 90%, AL4-1A1 65%.

After reviewing the serum stability results and consulting the relevant experts, we decided that two of the three lead compounds are qualified for mice assay. Prior to mice assay we conducted ex vivo cell assay to check the inhibition of TNF-α secretion from mice macrophages.

The three lead molecules were tested ex vivo for their ability to block TNF-α release from mice macrophage cells (RAW 264.7 cells) when exposed to a strong inflammatory stimuli (Lipopolysaccharide (LPS) from E. coli). We have chosen to begin with a cell inhibition assay because these results strongly correlated with success in live animal models. In addition, this assay allows us to test a wide range of inhibitor concentrations efficiently in a complex biological system before using animal models.

Upon LPS stimulation, we measured the concentration of soluble TNF-α secreted in cell supernatant using anti-TNF-α ELISA (enzyme linked immuno-sorbent assays). LPS treatment with no drug is considered endogenous or 100% TNF-α release. Concentration of endogenous TNF-α release is then compared to TNF-α release in the presence of our drug at various concentrations. The well established broad TACE inhibitor GM6001 (Galardi et al., 1994), served as positive control for the assay. GM6001 failed in clinical (reason un known), yet was tested successfully in cell assay (Solorzano et al).

LPS induced cells were co-administrated with 0-10 μM of AL24-2A1, AL4-1A1 or AL-AL-Rev1 and 10μM GM6001 for 0-24 hours. At the following time points (3, 6, 9 and 24 hours) the cell supernatant was collected and TNF-α levels were measured. Figure 1 graphically displays the TNF-α release in the presence of our TACE inhibitor AL24-2A1, AL4-1A1 or AL-AL-Rev1, upon LPS stimulation of mice macrophage cells. From these results, it is clear that both AL24-2A1 and AL4-1A1 reduce TNF-α release by more than 50%, compared to endogenous levels. Moreover, both drugs work in the picomolar range. While GM6001 performs as well as our drugs, it does so in 100-10000 times higher concentration. Important to note, AL4-1A1 retains its activity 9 hours after administration, and this is before any formulation or drug modification. Taken together, these results indicate that AL24-2A1 and AL4-1A1 are potent inhibitors and promising drug candidates for the upcoming animal tests. AL-AL-Rev1 is les potent and reduces TNF-α release not more than 30% after 3 hours and almost looses activity after 6 hours, while GM6001 still retains its activity after 6 hours, reducing TNF-α in ~30%. These results do not yet exclude AL-AL-Rev1 from being a drug since it works at low concentrations. All drugs showed no activity at time longer than 9 hours thus data not indicated. Yet 3-6 hours half life for a lead compound is a good starting point for further drug development.
Figure 1: Soluble TNF-α cell supernatant concentration upon co-administration of LPS with AL24-2A1 (a) AL4-1A1 (b) and AL-AL-Rev1 (c) at 3, 6 and 9 hours.

The three lead compounds, AL24-2A1, AL4-1A1, and AL-AL-Rev1, were successfully tested for toxic effects on mice in 0.05-25 mg/kg concentrations range. In view of parameters tested: weight, appetite, general behavior, movement, fur ect'; none of the inhibitors had toxic effect on the animals comparing to PBS vehicle control.
AL4-1A1 was successfully S.C tested for efficacy against collagen-induced arthritic (CIA (Zhang et al., 2004)). This mouse model designed to test the effect of tested drug on CIA mice that mimics the human RA. Mice are induced with calf collagen for joint arthritis. After second induction (21 days from the first collagen injection), they develop the disease and disease clinical score is determent according to disease severity. At first experiment (figure 2) AL4-1A1 was tested only at prophylactic model (daily injections from day 1 of the experiment till its end). AL4-1A1 at concentration of 1μg/mouse, significantly reduced the disease score by 30-35% (figure 2). Annova statistic showed that after day 31 (day count starts from first collagen injection, and day 24 is the first detection of disease score) the disease score was significantly reduced. At the second experiment (figure 3) we included therapeutic group (injections only from clinical score appearance) and lower dosages than 1ug/mouse in order to determine the therapeutic window of our drug. We also included a positive control, wide-range TACE/MMPs inhibitor – GM6001.

We got slightly better results with 0.1 μg/mouse and 0.01 μg/mouse retained the disease thus our therapeutic window lies at 0.1-1 μg/mouse. The therapeutic group worked and reduced the disease clinical score at ~30% at 1μg/mouse, proving the drug ability to cure and halt the disease (figure 3). Both prophylactic and therapeutic groups performed better that the positive control GM6001 and at much lower doses.

Comparing to known drugs tested in CIA mice model such as Methoxetrate, Enbrel and Ankinra (table 3) 30-35% disease score reduction is excellent starting point. Thus Allosterix has completed in vivo proof of concept in one of the three anti-TACE drugs.

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**Figure 2:** Clinical score of CIA (collagen-induced arthritis) mice from disease onset (day 24). Mice groups are of 10 items as follows: PBS = vehicle negative control, Dexa = 160μg/mice Dexamethazone, 1 = 1 μg/mice AL4-1A1. Statistical analysis was performed using Annova followed by Tukey Cramer tests.
Figure 3: Clinical score of CIA (collagen-induced arthritis) mice from disease onset (day 24). Mice groups are of 10 items as follows: PBS = vehicle negative control, AL4-1A1 0.1 μg/mice, AL4-1A1 1 μg/mice - therapeutic administration, GM6001 positive control – broad range TACE/MMPs inhibitor. Statistical analysis was performed using Annova followed by Tukey Cramer tests.

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Company</th>
<th>Reduction [%] of CIA score</th>
<th>Drug concentration</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methotrexate</td>
<td></td>
<td>~58% at prophylactic mice model (lower effect at therapeutic model)</td>
<td>35 mg/kg</td>
<td>Fiehn et al, Rheumatology, Vol 43, 1097-1105, 2004</td>
</tr>
<tr>
<td>Enbrel</td>
<td>Wyeth</td>
<td>~35% therapeutic mice model</td>
<td>2.5 mg/kg</td>
<td>Wooley et al, The Journal of immunology, Vol 151, No. 11, 6602-6607, 1993</td>
</tr>
<tr>
<td>Anakinra</td>
<td>Amgen</td>
<td>~70% prophylactic mice model</td>
<td>5 mg/kg</td>
<td>Bendele et al, Arthritis &amp; Rheumatism Vol 42, No. 3, 498–506, 1999; FDA August 2001 document</td>
</tr>
</tbody>
</table>

Table 3: Clinical score reduction by known approved RA drugs that were tested in CIA mice model
**Pathologies Affected by TACE inhibition**

**Arthritis**

In models of arthritis, TNF-α is a critical in disease initiation and shown to mediate joint damage and destruction. A common feature of inflamed tissue is an elevation in TNF-α concentrations. It stands to reason that a reduction in circulating TNF-α levels will disrupt the signaling cascade responsible for chronic inflammation and subsequent tissue damage. The success of anti-TNF-α agents provide evidence that TNF-α disruption is an effective means of altering or even reversing disease progression.

Promising results from phase II clinical trial with BMS' selective TACE inhibitor, BMS 561392, revealed a 2.8 mg/kg dose was more effective than Remicade® at reducing inflammation and a 10.5 mg/kg dose worked as well as Enbrel® (Grootveld and McDermott 2003). This supports the hypothesis that TACE inhibition is as effective as anti-TNF-α treatments at alleviating and altering the progression of rheumatoid arthritis (RA).

**Rheumatoid arthritis (RA)**

Rheumatoid arthritis, which affects approximately 1% of the US population, is a serious and chronic autoimmune disorder directed at the joints causing painful swelling and tissue destruction. It can be a disabling condition and can lead to substantial loss of function. In the early stage of the disease, joints are infiltrated by immune system cells, which continually signal to one another and initiate the self-perpetuating, chronic inflammation.

The primary goal of RA treatment is to control joint damage and prevent the loss of function. Treatment is centered on NSAIDs for pain relief and disease modifying anti-rheumatic drugs (DMARDs) to reduce the rate of bone and cartilage damage. Rheumatologists consider methotrexate to be first-line treatment due to its efficacy and relatively good safety profile (Saag et al., 2008). Methotrexate is considered the most tolerated of the DMARDs, and if it does not control arthritis initially, it is prescribed in combination with other DMARDs such as sulfasalazine (Azulfidine®, Santen), leflunomide (Arava®, Sanofi-Aventis) and the anti-malarial drug, hydroxy-chloroquine. Even with optimal use of DMARDs, the outcome for many patients with RA includes severe functional decline and considerable adverse side effects. Approximately 50% of patients will show an improvement in disease score and radiological joint improvement.

Biologic anti-TNF-α agents, used with methotrexate, are the next course of treatment when all other DMARDs fail. TNF-α antagonists were the first of the biological disease modifying to be approved for the treatment of RA, currently three TNF-α antagonists approved by FDA approval for RA are (listed in order of their approval for RA); etanercept (Enbrel®), infliximab (Remicade®), and adalimumab (Humira®). Success of the TNF-α directed biologics has paved the way for cytokine antagonists such as the IL-1 directed anakinra (Kinereit®), as well as immune cell blockers, including the B-cell directed rituximab (Rituxan®) and T-cell blocker abatacept (Orencia®) (Moss et al., 2008).
**Psoriasis and Psoriatic Arthritis**

Psoriasis is the chronic inflammation of the skin cells which creates itchy scales and plaques all over the body. Although these symptoms are not life threatening, they negatively impact social and emotional quality of life. TNF-α is thought to be the primary mediator of these events; though the inflammatory trigger is still unclear. Psoriatic arthritis (PA) is a debilitating disease that targets the hand and foot joints. First line therapy is methotrexate and if the condition continues to worsen, Remicade® can provide symptom relief (Woolacott *et al*., 2006). Unfortunately, this form of arthritis is not as responsive as RA to routine treatment and benefits of treatment usually subside after use. There is a particular unmet need for effective, long-term therapies for psoriasis and PA. We predict that a TACE inhibitor, given daily over a period of years, would be a cost effective means of controlling symptoms and reducing painful itching and scaling.

**Ankylosing spondylitis**

Ankylosing spondylitis (AS) is the chronic inflammation of spinal vertebrae which can lead to the fusion of vertebrae, a debilitating and irreversible condition. The pathogenesis of ankylosing spondylitis is not well understood, however the release of TNF-α has been proposed as the inflammatory initiator.

Of critical importance, 60% of AS patients will develop an irritable bowel inflammation, similar to Crohn's disease and treatment with Remicade® has been shown to reduce this percentage significantly (Brawn *et al*., 2007). This discovery demonstrates the consequence of excessive TNF-α and highlights the long-term benefits of a TACE inhibitor.

Currently there is no standard or effective therapy for AS. Conventional management is composed of physiotherapy, NSAIDs and DMARDs for extreme symptoms of pain and stiffness. Unfortunately, treatment with DMARDs for AS patients has been disappointing. The conclusions of the Cochrane review on the use of SSZ in patients with AS are as follows: “across all AS patients, SSZ demonstrated some benefit in reducing inflammation and easing morning stiffness, but no evidence of benefit in physical function, pain, or spinal mobility. Biologics have the proven effective in reducing pain and disease score, however do not appear to alter the disease progression” (Zochling *et al*., 2005).

**Inflammatory Bowel Disease**

Inflammatory bowel disease (IBD) affect millions of people worldwide, currently 2.8 million are diagnosed with ulcerative colitis (UC) or Crohn’s disease (CD) in the US alone. IBD increases patient morbidity, decreases quality of life and increases patient susceptibility to colorectal cancer. The incidence of IBD is rising in developing countries around the world. Currently the global market is estimated at $16 billion and this is set to continue to rise.

TNF-α and oxidants are the key inflammatory factors involved in tissue injury in both ulcerative colitis and Crohn’s disease (Mizoguchi *et al*., 2002; Mutlu *et al*., 2003). Currently two key steps of IBD pathogenesis have been identified: hyperpermeability of the gut to bacterial products and an abnormal immune response to these products (Forsyth *et al*., 2007). Both of these events are associated with TACE activity. In fact, intestinal hyperpermeability was
successfully blocked by specific inhibitors of the EGFR, TACE, transforming growth factor (TGF-α), and extracellular signal-regulated kinase 1/2 (ERK1/2) (Forsyth et al., 2007).

TACE is functionally active in the human colon and its activity is increased in ulcerative colitis, which raised interest in testing inhibitors in models of IBD. In the intestine of IBD animal models, MMP/TACE inhibitors were able to eliminate inflammation in animal models of IBD (Brynskov et al., 2002).

Anti-TNF-α therapies have been shown to be effective in the treatment of IBD and demonstrate the pathogenic role of TNF-α in IBD as well as the therapeutic gain of targeting TNF-α (Maksymowych 2007). One of the mechanisms underlying the deleterious effects TNF-α in IBD is increased intestinal permeability in response to oxidative stress. It has been shown that this oxidant-induced intestinal hyperpermeability can be blocked by specific inhibitors of TACE (Forsyth et al., 2007).

**Ocular inflammatory diseases**

At the last three years, TACE was linked to ocular inflammatory diseases. Augmented levels of inflammatory cytokines, specifically tumor necrosis factor-α (TNF-α) have been implicated in several ocular diseases such as Uveitis (Kota 2007) and Glaucoma (Frederic 2009). Glaucoma can lead to blindness if not treated and Uveitis is a major cause of severe visual impairment that accounts for 10–15% of all cases of total blindness in the US.

**Cancer**

TACE has been shown to act upon the epidermal growth factor receptor (EGFR), a receptor linked to many cancer pathobiologies. Inhibition of EGFR signaling has been shown to be an effective means of treating breast cancer. In a breast cancer cell line, use of a TACE inhibitor prevented the release of EGFR ligands, such as TGF-α, and was able to revert the malignant phenotype (Kenny 2007). This establishes a rationale for testing TACE inhibitors against malignancies involving EGFR.

**Asthma**

A positive correlation between TNF-α levels in the airways and severity of asthma has ignited interest in TACE inhibitor for refractory asthma sufferers (Howarth et al., 2005). Patients with severe and refractory asthma suffer from numerous complications, some of which can be fatal. Treatment options are limited and it is unclear why they are "resistant" to traditional glucocorticoid therapy. Various mouse models of asthma deficient in TNF-α, or TNF-α receptors, or treatment with anti-TNF-α antibody resulted in an attenuation of antigen-induced airway hyper responsiveness and airway inflammation. Thus, TACE inhibition is a promising therapeutic option for severe, refractory asthma cases.
**Ischemic Stroke**

Stroke is the third leading cause of death and the leading cause of permanent disability in western countries. The incidence of stroke is expected to increase in the foreseeable future due to the ageing population. The only FDA-approved therapy for stroke during the first 3 hr after the disease onset is recombinant tissue plasminogen activator (rt-PA).

Inflammation has been known to play a critical role in the induction and development of stroke and TNF-α is associated with ischemic stroke. Anti-TNF-α biologics have been shown to be effective in reducing the disease symptoms in various pre-clinical stroke models. However due to the aforementioned limitations of biologic therapeutics, there is a need for small molecule TNF-α inhibitors. There is data which strongly suggests that TACE/MMP inhibitors have great therapeutic potential and may be valuable alternatives in treating stroke in the clinic (Lovering and Zhang, 2005).

**Current Therapeutic Landscape**

The first line treatment for rheumatoid arthritis is methotrexate due to its efficacy, low cost, and relatively good safety profile. If methotrextrate is not effective on its own, physicians will often combine this drug with other well established disease modifying drugs, such as sulfasalazine and/or leflunomide. In 50% of patients, the disease will not show signs of resolution and these patients will qualify for biologic therapy, agents directed against TNF-α and taken in combination with methotrextrate. Biologic DMARD agents show good efficacy and manageable safety profiles; however the cost of these agents is prohibitive due to drug formulation and route of administration. However the success of biologic agents provides solid evidence for the benefits of modulating soluble TNF-α levels as a means of reversing disease progression for optimal clinical outcome.

In fact, clinical trials clearly demonstrate that early treatment with biologic agents greatly improves the overall clinical outcome, in terms of speed and duration, and many companies suggest these agents should be prescribed sooner by rheumatologist. Unfortunately, with the current costs of these drugs, the health system can not support this line of therapy for all patients and must be reserved for non-responsive cases. We believe our TACE inhibitor will be the most cost effective means of regulating TNF-α levels and affordable enough to be used as a first line treatment for all patients.

Psoriasis and ankylosing spondylitis patients do not show signs of disease modification with current battery of DMARDs, and these patients are in desperate need of new therapies. In terms of symptom management, Remicade® is the most effective at reducing inflammation; however results do not persist after treatment. The second most effective treatment for pain and swelling is methotrextrate in combination with ciclosporin, followed by Enbrel® and then Oenerica®. Based simply on the cost of these therapies, it is evident that methotrextrate and ciclosporin is the most logical first line therapy, at $85-364 per year. In fact, methotrextrate and ciclosporin generates similar benefits to Remicade® ($21,200 per year) and better results than Enbrel® ($9361 per year), at literally a fraction of the cost. The great divide in therapeutic cost underlines the demand for effective, orally available, small biomolecules with drug-like properties, which can offer the same efficacy and better safety profiles than biologic agents, at a fraction of the price.
<table>
<thead>
<tr>
<th>Drug</th>
<th>Target</th>
<th>Rheumatoid Arthritis</th>
<th>Psoriasis</th>
<th>Ankylosing Spondylitis</th>
<th>Crohn’s</th>
<th>Ulcerative Colitis</th>
<th>Cost per Year</th>
<th>Sales US 2007</th>
<th>Adverse Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of onset</td>
<td>~40 years</td>
<td>~30 yrs 1:4 yrs 18yrs</td>
<td>~30 yrs</td>
<td>~30 yrs</td>
<td>~30 yrs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prevalence (in US)</td>
<td>1% or 2.1M</td>
<td>0.13% or 350G</td>
<td>0.18% or 500G</td>
<td>Less than 0.1%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSAID</td>
<td>COX 1/2</td>
<td>Pain relief</td>
<td>Pain relief</td>
<td>First Line</td>
<td>n/a</td>
<td>n/a</td>
<td></td>
<td>Generic</td>
<td>Cardiovascular risk</td>
</tr>
<tr>
<td>sulphasalazine Santen Pharma</td>
<td>Used after MTX</td>
<td>Used after MTX no DMARD</td>
<td>*</td>
<td>*</td>
<td>?</td>
<td>GI events</td>
<td></td>
<td></td>
<td>Allergy to sulphur</td>
</tr>
<tr>
<td>Arava® leflunomide Sanofi- Aventis</td>
<td>T cells</td>
<td>Used after MTX</td>
<td></td>
<td>$543</td>
<td>Generic</td>
<td>Liver Toxicity</td>
<td></td>
<td></td>
<td>Cost, IV events antibodies</td>
</tr>
<tr>
<td>Enbrel® etanercept Wyeth/ Amgen</td>
<td>TNF-α</td>
<td>#2 effective</td>
<td>#3 effective</td>
<td>#2 effective, no DMARD</td>
<td>?</td>
<td>?</td>
<td>$9,361</td>
<td>3200M</td>
<td>Cost, increase in TB/sepsis</td>
</tr>
<tr>
<td>Remicade® infliximab Schering-Plough</td>
<td>TNF-α</td>
<td>#3 effective</td>
<td>#1 effective</td>
<td>#1 effective, no DMARD</td>
<td>*</td>
<td>*</td>
<td>21,211</td>
<td>2900M</td>
<td>Upper respiratory TB</td>
</tr>
<tr>
<td>Humira® adalimumab Abbott Lab.</td>
<td>TNF-α</td>
<td>*</td>
<td>*</td>
<td>#3 effective, no DMARD</td>
<td>17,881</td>
<td>2900M</td>
<td></td>
<td></td>
<td>Respiratory infections COPD</td>
</tr>
<tr>
<td>Ocrecia® abatacept BMS</td>
<td>T-cells</td>
<td>*</td>
<td></td>
<td></td>
<td>$15,15</td>
<td>312M</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other FDA approved drugs</td>
<td>Hydroxychloroquine RituXan® Celebrex® Ciclosporin with MTX, Raptiva® Physiotherapy Tysabri® Colazad® Cimzia®</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Overview of current therapeutic landscape for immune-mediated disorders detailing; prevalence, therapeutic options, treatment guidelines, and 2007 sales.

Where # is effectiveness rating from cross-analysis of randomized clinical trials, * drug is used for this condition, ′ drug used only if previous treatments are not effective.
Future Market Trends

Within the next five years, we will see a boom in biologic agents geared towards mediating arthritis and a host of other autoimmune conditions. Many are in Phase III of development and appear poised to take a portion of Enbrel® and Remicade®’s multi-billion dollar market share. Novartis is conducting Phase II trial for an IL-1 directed agent, and Roche's IL-6 agent is currently in Phase III. GSK has a B-cell modulator in Phase II development and Takeda currently has two targets in Phase II directed against T-cells and p38. Wyeth and Trubion are trying to build on the success of TRU-015, currently testing a new EGFR-agent called ERB-014 (Table 5).

However, we feel these additions to the market will flood the biologics arena but fail to address current market demand for safer and cost effective treatments. Should a drug with these properties come to market, it would become an economic and efficacious means of treating all patients regardless of disease severity.

Table 5. Overview of preclinical and clinical results of biologic agents directed at cells and mediators involved in the inflammatory cascade for the treatment of immune-mediated disorders.

<table>
<thead>
<tr>
<th>Company</th>
<th>Drug</th>
<th>Target</th>
<th>Clinical</th>
<th>Dose</th>
<th>Status</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wyeth</td>
<td>ERB-041 Epidermal Receptor</td>
<td>RA/ PA IBD</td>
<td>PII with MTX</td>
<td>Oral 1xmonth</td>
<td>Start: Feb 05 Still Active</td>
<td>CT.gov</td>
</tr>
<tr>
<td>Wyeth Trubion</td>
<td>TRU-015</td>
<td>CD20 on B cells RA</td>
<td>PII with MTX</td>
<td>2 Shots</td>
<td>Start: Mar 08 - June 2011</td>
<td>CT.gov</td>
</tr>
<tr>
<td>Wyeth Trubion</td>
<td>SBI-087</td>
<td>CD20-directed</td>
<td>PI</td>
<td>IV</td>
<td>Still Active</td>
<td>CT.gov</td>
</tr>
<tr>
<td>Takeda</td>
<td>TAK-715</td>
<td>p38</td>
<td>PII with MTX</td>
<td>Oral</td>
<td>Sept 2005</td>
<td>Nature CT.gov</td>
</tr>
<tr>
<td>Takeda</td>
<td>TAK-783</td>
<td>T cell</td>
<td>PII</td>
<td></td>
<td>Start 2007 March 2009</td>
<td>CT.gov</td>
</tr>
<tr>
<td>BMS</td>
<td>BMS-188667 (Orencia or apatacept)</td>
<td>Anti-TNF RA, JA</td>
<td>Several PIII underway</td>
<td></td>
<td>Ongoing</td>
<td>CT.gov</td>
</tr>
<tr>
<td>GSK</td>
<td>3152314A (ofatumumab)</td>
<td>B-cell activation RA</td>
<td>PII</td>
<td>Shot</td>
<td>Oct 2008</td>
<td>CT.gov</td>
</tr>
<tr>
<td>Novartis</td>
<td>ACZ885 (canakinumab)</td>
<td>Targets IL-1 RA</td>
<td>PII</td>
<td>IV and subcut.</td>
<td>Active November 2008</td>
<td>CT.gov</td>
</tr>
<tr>
<td>Roche</td>
<td>tocilizumab (Actemra)</td>
<td>IL-6 RA / Inflam</td>
<td>2x PII with MTX 44/38 % improvement for non responders to DMARDs</td>
<td>One shot per week</td>
<td>Will complete in June 09</td>
<td>ACR Nov 07 meeting</td>
</tr>
</tbody>
</table>
Conclusion
It is our contention that a small, allosteric-based inhibitor will dramatically impact the current treatment strategies for inflammation-based disorders. We believe a TACE inhibitor could be used as a first line DMARD treatment for all patients presenting TNF-α related diseases, regardless of severity, due to the low cost of the drug. This confidence stems from the success of TNF-α antagonist, which provided a proof of concept that TACE inhibition is an effective means of modulating TNF-α levels. A decrease in TNF-α results in a reduction in inflammation and can modify the rate of disease progression and tissue destruction.

The current unmet need for effective drugs is exacerbated by great divide between the cost of traditional DMARDs and biologic agents. This effectively means that patients must first try all cost effective therapies prior to biologic agents, although evidence indicates that patient outcome would improve if these expensive therapies would be given at an earlier time point. The patient and market demand for effective, orally available, small peptides with drug-like properties, which can offer the same efficacy and better safety profiles than biologic agents, at a fraction of the price, is at its peak. This divide between affordable drugs and biologic agents will only increase with the introduction of more than 10 new biologic agents within the next 5 years.

Allosterix has created a TNF-α modulator that is orally available and inexpensive to manufacture. These two properties will allow us to meet the current demands and help millions of people each year that suffer from TNF-α mediated diseases. There is promising evidence that other areas of medicine might benefit from this mechanism of action as well. TACE inhibition may be a viable therapeutic option for severe asthma sufferers or could be used as a prophylactic for at risk patients in hospitals, to mitigate the inflammatory response during septic shock (Horiuchi et al., 2007). TACE inhibition has been sought after for years, and we at Allosterix, have developed a novel approach to targeting TACE in a manner that confers unprecedented selectivity and affinity for its target. We invite you to become part of our exciting discovery.
References:


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4-quinolinyl) methoxy[ phenyl]-2-oxopyrrolidinyl]-N-hydroxy-4-methylpentanamide)), a Potent and Selective Inhibitor of Tumor Necrosis Factor α-Converting Enzyme in Rodents, Dogs, Chimpanzees, and Humans. *Drug Metabolism and Disposition* **2007** 35(10):1916-25.


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