The Immunological Basis for Treatment Resistance to Anti-TNF Treatments

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Rationale and Hypothesis

Anti-TNF treatments such as etanercept are one of the most effective treatments available for psoriasis, but treatment response can vary substantially and a subgroup of patients may show minimal or no response even at high doses, despite having otherwise identical clinical features. The biologic basis for the resistance to anti-TNF treatment is unknown but several separate lines of evidence indicate that it might be due to cross-regulation between tumor necrosis factor (TNF)-α and the type I interferon (IFN), IFN-α.

The major source of type I IFNs are plasmacytoid dendritic cells (pDCs). pDCs are rare in healthy skin but are often increased in psoriatic skin (4) and have been implicated in the pathogenesis of psoriasis, particularly in triggering plaque development, which has been shown to be dependent on IFN-α production in a xenograft mouse model. Type I IFNs inhibit TNF-α production from monocyte-derived inflammatory cells and anti-TNF pretreated pDCs have about a three-fold increase in IFN-α production. Furthermore, TNF blockade is consistently associated with an increased IFN-α signature in blood cells. In stark contrast, and consistent with the cross-regulation between these two cytokines, TNF-α inhibits the release of IFN-α from pDCs as well as generation of pDCs from hematopoietic precursors.

The clinical evidence supporting this phenomenon has grown over the past few years. The induction or exacerbation of psoriasis during anti-TNF therapy, which has now been reported in several hundred patients (9, 10) is one such example, and may occur in patients treated for inflammatory conditions other than psoriasis. The psoriatic lesions of these patients have a greatly increased type I interferon signal, as determined by increased tell-tale Myxovirus Resistance Gene A (MxA) expression, compared with control psoriasis samples and similar observations have been made in patients exposed to the Toll-like receptor (TLR)-7 agonist imiquimod. Imiquimod is a very effective inducer of type I interferon production by pDCs (15) and has been shown to exacerbate psoriasis both at the treated area as well as distant skin sites. Similarly, to anti-TNF exacerbated psoriasis, this reaction is accompanied by influx of pDCs and increased MxA expression. Interestingly, but in line with the observations above, this reaction appears to be exacerbated in patients on anti-TNF treatment. This cross-regulation is likely to extend beyond psoriasis as diseases characterized by increased type I IFN signal are frequently exacerbated or triggered by TNF treatments, including lichenoid skin reactions and the increased frequency of positive anti-double stranded DNA antibody titers and less commonly clinical systemic lupus erythematosus, which are both reversible upon discontinuation of treatment.

We have demonstrated that the inflammatory and cytokine network in chronic plaque psoriasis is heterogenous with large variation in the strength of the type I

IFN-α and TNF-α signals between different patients. Thus, about 35-40% of patients show an enriched IFN-α signature in lesional psoriatic skin, which, perhaps coincidentally, is around the same ratio of patients showing limited response to etanercept therapy. Our analysis of a small cohort of patients (n=15) treated with etanercept indicated that those patients with increased IFN-α signal had poorer response to therapy and that the strength of the IFN-α signal was the main predictor of treatment response.

These observations form the basis of our hypothesis **that the balance between type I IFN and TNF determines the response to anti-TNF treatment**. The goal of the proposed study is to address this hypothesis and demonstrate that the strength of the type I IFN signature in psoriatic skin is the major determinant of the clinical response to anti-TNF treatment.

Proposed experiments

Purpose: Determine the strength of the type I interferon and TNF signal in psoriatic skin prior to and during treatment with etanercept and correlate with degree of clinical improvement.

Psoriatic patients will receive 100 mg etanercept per week (2 separate single-use pre-filled 50 mg subcutaneous injections taken on two separate days) for 3 months. Inclusion and exclusion criteria will be identical to the previous EASE trial (with the exception of the highlighted items), as follows:

Inclusion:

1. At least 18 years of age at screening.
2. Clinically stable moderate to severe plaque psoriasis at screening and baseline.
3. Subject must be:
    - A man **or**
    - A woman who is surgically sterile or at least 3 years postmenopausal **or**
    - A woman of childbearing potential who has had a negative pregnancy test within 7 days before the first dose of study drug.
4. If the subject is sexually active, (s)he must agree to use a medically acceptable form of contraception during screening and throughout the study.

Exclusion:

1. Grade 3 or 4 adverse events or infections within 28 days before screening, or between the screening visit and drug initiation.
2. Active or chronic infection within 4 weeks before screening visit, or between the screening and baseline visits.
3. Evidence of skin conditions other than psoriasis that would interfere with the evaluations of the effect of study medication on psoriasis.

4. Use of oral psoralen with ultraviolet A (PUVA), oral retinoids, cyclosporine, alefacept, or any other systemic anti-psoriasis therapy within 28 days study drug initiation.
5. Use of ultraviolet B (UVB) therapy, topical steroids at no higher than moderate strength, topical vitamin A or D analog preparations, or anthralin with 14 days of study initiation.
6. Prior or concurrent use of cyclophosphamide therapy
7. Concurrent sulfasalazine therapy.
8. Known hypersensitivity to Enbrel® (etanercept) or any of its components or known to have antibodies to etanercept.
9. Current enrollment in any other investigational device or investigational drug trial(s), or receipt of any other investigational agent(s) within 28 days before baseline visit.
10. Use of any biologic drugs within 28 days of study drug initiation.
11. Concurrent use of Anakinra.
12. Severe comorbidities (diabetes mellitus requiring insulin; congestive heart failure (CHF) of any severity or myocardial infarction or cerebrovascular accident or transient ischemic attack within 3 months of screening visit; unstable angina pectoris, uncontrolled hypertension (sitting systolic BP <80 mm Hg or > 160 or diastolic BP > 100 mm Hg), oxygen-dependent severe pulmonary disease, history of cancer within 5 years (other than resected cutaneous basal or squamous cell carcinoma of the skin or in situ cervical cancer)
13. Known history of tuberculosis (TB), or previous positive purified protein derivative (PPD) test. Any mycobacterial disease or high risk factors for tuberculosis (TB), such as family member with TB, positive purified protein derivative (PPD) or taking anti-tuberculosis medication.
14. Known HIV-positive status or known history of any other immuno-suppressing disease.
15. Concurrent or history of psychiatric disease that would interfere with ability to comply with study protocol or give informed consent.
16. History of alcohol or drug abuse within 12 months of screening visit.
17. Latex sensitivity [Nota Bene: only applicable if they are using prefilled syringe or prefilled SureClick™ autoinjector presentations]
18. Exposure to hepatitis B or hepatitis C or to high risk factors for hepatitis B or C, such as intravenous drug use in patient.
19. Systemic lupus erythematosus, history of multiple sclerosis, transverse myelitis, optic neuritis or seizure disorder.
20. Use of a live vaccine 90 days prior to screening visit, or concurrent use of a live vaccine.
21. Any condition or circumstances judged by the patient's physician[or the investigator or medically qualified study staff] to render this clinical trial detrimental or otherwise unsuitable for the patient's participation.
22. History of non-compliance with other therapies.
23. Pregnant or nursing females.

24. Diagnosis of multiple sclerosis in first degree family relationship (parent, sibling or child)

Flow Chart of visits:

Visits	Screening	BL	Week2	Week 6	Week 12
ICF	X				
Med/Hx	X				
Con Meds	X	X	X	X	X
Biopsies		X	X	X	X
Inc/exc	X				
Screening labs (CBC, metabolic panel)	X				
TB Test*	X				
Blood		X	X	X	X
Dispense Drug		X	X	X	
Urine Pregnancy Test	X	X		X	
Photos		X	X	X	X
Injection Instruction		X			
Physical Exam	X	X			
PASI/PGA/BSA/ Itch Assessment**	X	X	X	X	X

*Subjects will be tested for TB at the screening visit and will return to research clinic 2 days later so that the area can be examined and the result documented.
**Subjects will be asked to verbally indicate their level of itch on a scale of 0 to 10 at each visit.

Adverse events will be reported in accordance with the guidelines of the Institutional Review Board (IRB) and the Food and Drug Administration (FDA). Concomitant medications will be recorded. All serious adverse events, regardless of causation, will be reported to the IRB and Amgen Global Safety (see below) within one working day. All serious and medically significant adverse events considered related to etanercept by the investigator will be followed until resolved or considered stable. Unexpected and fatal or life-threatening events will be reported to the FDA by phone or FAX within 7 calendar days. Written reports will be sent to the FDA within 15 calendar days for any serious unexpected adverse events for which there is reasonable possibility that the event may have been caused by the study drug. Any pregnancies occurring on study must also be reported to Amgen Global Safety (see below). Within 24 hours of FDA notification, a copy of all FDA submitted reports will be sent to Amgen by facsimile or mail at the following address:

Amgen Global Safety
c/o Amgen
One Amgen Center Dr.
Mail Stop 17-1-A
Thousand Oaks, CA 91320-1799
Fax: 1-888-814-8653

At the screening visit, subjects will have a TB test performed, as well as skin exams, physical exams, medical history review, etc. as documented in the study flow sheet to determine if they meet the entrance requirements.

Peripheral blood and biopsies will be obtained from 50 psoriatic patients with active lesions, who have not received any systemic treatment for four weeks, or any topical treatment other than bland emollients for two weeks. Peripheral blood mononuclear cells (PBMC) will be isolated from blood (approximately 4-6 tubes) taken from psoriatic patients prior to treatment and at every visit of etanercept treatment (see flow chart). The isolated PBMCs will be cryopreserved and stored in liquid nitrogen, or used immediately for experiments. Serum will be stored for future cytokine measurements and levels of anti-nuclear antibodies (ANA) by ELISAs. Cellular staining will be determined by twelve-color BD LSR II flow-cytometer. Two biopsies will be taken at baseline (uninvolved and lesional psoriatic skin) and at each visit (lesional only) at week 2, 6 and 12. Gene and protein expression may be analyzed in whole-biopsies, or certain cell populations may be isolated from biopsies to perform flow cytometry, gene expression studies, protein expression studies, and other laboratory studies.

1. Demonstrate that the strength of type I IFN signature in psoriatic skin predicts treatment response.

Fifty individuals with moderate to severe psoriasis with high dose etanercept (50mg twice weekly), and the composition of the lesional psoriatic cytokine network will be tracked at the onset (day 0), early (week 2), during the middle (week 6) and at the end of treatment (week 12). Serum samples and PBMC isolation will be done at each time-point for later analyses. Biopsies of uninvolved and lesional skin will be performed before initiation of treatment and then lesional biopsies taken at week 2, week 6 and week 12. Skin will be anaesthetized with 1% lidocaine with epinephrine and 6mm punch biopsies will be taken. Photographs of patients will be obtained and the anatomical location of biopsies recorded. Biopsies will be bisected, with one-half snap frozen in liquid nitrogen and stored at -80° until RNA and protein processing and the other half fixed in 4% formalin for paraffin embedding and immunohistochemistry. Processing will be performed as previously described by our team and only high quality RNA, as determined by Agilent 2100 Bioanalyzer will be used .

Next-generation sequencing of RNA (RNAseq) will be performed on the samples according to Illumina's protocol using 2µg of RNA. Immunohistochemistry will be performed to validate findings with MXA staining used to confirm presence of type I interferon signal. H&E staining will be used to assess the degree of epidermal hyperplasia and response to treatment and immunohistochemical detection of keratin 16, CD3, CD11c, CD163, Ki67 used to validate the degree of histologic response in context of the clinical response. QRT-PCR will be performed for selected gene transcripts to validate the findings of RNAseq.

Analysis/Statistics: RNAseq reads will be mapped using the TopHat algorithm and gene expression levels will be expressed as reads per kilobase of region per million mapped reads (RPKM). Based on prior experience, we estimate that we will obtain around 3 gigabases of sequence for each sample. Inflammation profiles and cytokine signature scores will be calculated as described by our group[1-3]. Estimating that around 50% of patients will achieve a 75% reduction in Psoriasis Area and Severity Index (PASI) (PASI75) at 12 weeks; this should allow us to determine if effective treatment is associated with complete neutralization of the TNF signal in lesional psoriatic skin and elucidate the effect of the strength of the type I IFN signal on treatment response. For our statistical analyses, we are interested in one outcome variable (PASI score) and two continuous covariates (RNAseq-generated TNF-α signal and IFN-α signal). We anticipate that these data will be analyzed in two ways.

(1) First, we will test whether temporal change in PASI score is significantly associated with baseline TNF-α signal, baseline IFN-α signal, and/or an interaction between these two baseline signals. Along these lines, a cumulative score reflecting change in PASI during the course of etanercept treatment will be calculated for each patient. This score will then be treated as a continuous response variable, and we will use robust linear modeling to test whether scores are significantly associated with baseline TNF-α signal, baseline IFN-α signal, and/or an interaction between these two signals. In addition, we expect that it will be possible to unambiguously classify 30% of patients as non-responders, with an equal percentage showing clear improvement in PASI score, and the remainder showing an intermediate response. Accordingly, we will also categorize patients according to improvement in PASI score (i.e., responders, intermediate-responders, and non-responders), and use cross-validation to calculate AUC statistics to directly quantify the capacity of baseline TNF-α and IFN-α signals to predict patient response status. Based upon preliminary data (n = 15 patients), we anticipate that improved PASI score will be associated with a heightened baseline TNF-α signal and weakened baseline IFN-α signal. The proposed study design will allow us to not only confirm this expectation, but will provide increased power to assess a possible TNF-α/IFN-α interaction effect, and to better characterize quantitative thresholds associated with response and non-response.

(2) Second, we will test whether TNF-α and/or IFN-α signals are significantly altered during the course of etanercept therapy, and whether such changes differ between subjects with different PASI response profiles. In these analyses, TNF-α or IFN-α signal will be treated as a response variable in a univariate repeated measures analysis of variance, with subject and time as covariates. In this framework, a shift in TNF-α or IFN-α signal during the course of etanercept treatment will be indicated by a significant time effect. In follow-up analyses, models will include 0-1 covariates indicating specific PASI response profiles (e.g., non-responder, intermediate-responder, or responder), which will allow us to test whether temporal shifts in TNF-α or IFN-α signals differ according to the effectiveness of etanercept treatment (i.e., by evaluating interaction effects involving time and 0-1 covariates denoting PASI response patterns). We anticipate that etanercept treatment will more strongly attenuate TNF-α signals in responders compared to intermediate-responders or non-responders (possibly due to disparities in baseline TNF-α and IFN-α signals). Conversely, we expect that non-responders will be characterized by high IFN-α signals, which remain high during the course of etanercept treatment. All analyses performed using PASI scores will be repeated using physician global assessment scores as the response variable, although we expect results from these two analyses to be consistent. With 50 patients recruited and treated, we estimate that we will have >95% statistical power to detect significant effects in the proposed design (Type I error rate of 0.05). Although the analysis will be primarily focused on TNF and IFN signatures, this dataset will also provide us with opportunities to determine contribution of other inflammatory cellular and cytokine contribution to the clinical response.

Anticipated Results: We expect that patients with strong IFN-α signature in psoriatic skin along with weak TNF-α signature will have minimal response to anti-TNF treatment, while patients with the opposite pattern, weak IFN and strong TNF signature, will have significant clinical improvement.