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The transfer of a laboratory based hypothesis to a clinically useful therapy: the development of anti-TNF therapy of rheumatoid arthritis

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The development of anti-TNF therapy is a key step forward in rheumatology as it is the first new therapy for based on investigating the molecular mechanisms of this disease. This chapter reviews how this discovery was made.

Key words: cytokine; therapy; antibody; anti-TNF.

Laboratory research is a relatively recent phenomenon, as judged by the growth of spending on laboratory research and by the number of publications. It is essentially a post-second-world-war-event, fuelled by the growth on spending by governmental agencies such as the National Institutes of Health (USA), Medical Research Council (UK) and related organizations over the rest of the western world, hugely supplemented by charitable and industrial funds. The key technical developments in tissue culture, molecular biology and immunology are all very recent (since the 1970s), as is their clinical counterpart, the randomized controlled clinical trial, as the arbiter of clinical efficacy.

The example we have been involved in for the past 15 years is a successful 'bench to bedside' development which brought a targeted biological therapy for rheumatoid arthritis (RA) into clinical practice. It could not have been initiated or come to fruition very much sooner as the key technologies and pathogenetic concepts had not matured.

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Rheumatoid arthritis is a systemic disorder chiefly localized to joints and associated with autoimmunity and chronic inflammation (although with some systemic features), affecting ~0.5–1% of the population.¹

THE EVOLUTION OF KNOWLEDGE-BASED THERAPIES

Immune

The association with HLA-DR4, the infiltration of synovium with abundant T lymphocytes (especially CD4⁺ cells) and the upregulation of local MHC class I and II^{2,3} (Figure 1) led to the concept in the 'mid-late' 1980s that CD4⁺ T cells were dominant drivers of RA⁴, leading to clinical trials of anti-CD4 antibody, with minimal success.⁵ While these failures may have been technical, due to inappropriate antibodies, dosing regimens, etc. schools of thought have cast doubt on the relevance of CD4⁺ T cells emerged.⁶

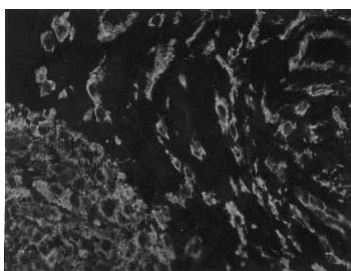
The discovery of rheumatoid factor as a diagnostic bio-marker of RA in the middle of the last century had heralded the era of its immune-mediated pathogenesis. Despite doubts about the role that antibodies might play in RA in the subsequent 50 years, preliminary clinical trials of an antibody targeting CD20 on B cells conducted in the past year have revived interest in the biology of B cells in RA.^{7–9}

Cytokines

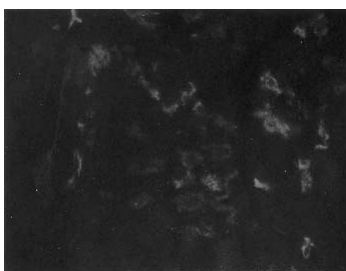
During the 1980s the molecular cloning of short-range protein mediators, known as 'cytokines', progressed rapidly, first with the interferons¹⁰, interleukin-1 (IL-1) (reviewed in Ref. 11) and interleukin-2 (IL-2) (reviewed in Ref. 12), and then with tumour necrosis factor (TNF)¹³ and lymphotoxin.¹⁴ The activities of many of these

WHY LOOK FOR CYTOKINES IN RHEUMATOID ARTHRITIS?

Upregulation of HLA-DR in rheumatoid synovium
(Klareskog, Wigzell etc. 1981/82)



Rheumatoid Arthritis



Osteoarthritis

Expression of HLA-DR on cells usually negative indicates presence of inducers = cytokines

Figure 1. Elevated levels of MHC class II molecule expression in rheumatoid arthritis compared to osteoarthritis: evidence from an immunohistochemical study.

mediators closely mirror the processes occurring in chronic inflammatory diseases, and so many laboratories set out to document which, if any, were expressed at sites of disease. The report of Fontana et al¹⁵ was the first to document the expression of interleukin-1 in rheumatoid synovial fluid and many other reports of cytokine expression rapidly followed^{16–18} (reviewed in Ref. 19). The expression of many pro-inflammatory cytokines in the synovium of joints with active RA—for example IL-1, tissue necrosis factor- α (TNF- α), GM-CSF and IL-6—with very closely related functional properties raised a question. Could a single cytokine be an effective therapeutic target, or would the blockade of a single cytokine leave others with similar properties to continue driving the chronic inflammation? This led multiple groups to abandon research in this field.

However, as the pro-inflammatory cytokines are present almost exclusively at times of 'stress' (immunological, infectious, physical) in trace amounts and have the capacity to activate multiple inflammatory pathways, they appeared to be just the type of rate-limiting molecules that might prove to be good therapeutic targets. Hence, we continued in our studies, and turned to analysing the cytokine regulation in human rheumatoid synovium.

Cytokine regulation in human rheumatoid synovium

The first experiments in this field in our laboratory were undertaken by Glenn Buchan, a postdoc from New Zealand, who had been evaluating cytokine expression in tissues at mRNA level, using Northern blots and slot blots (which used fewer cells). Buchan found that the time-course of IL-1 mRNA expression in rheumatoid synovial cultures was prolonged (Figure 2), compared with the short pulse of IL-1 mRNA expression, induced by stimulating leukocytes from normal blood.²⁰ The rheumatoid synovial cell

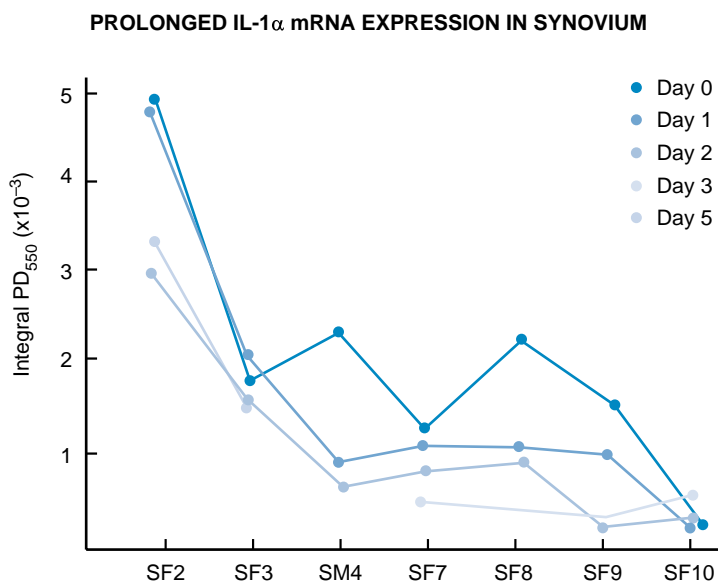


Figure 2. Slot blot analysis of IL-1 production by mononuclear cells from the joints of patients with RA. Cells were cultured in the absence of extrinsic antigen. Redrawn from Buchan G et al (1988, *Clinical and Experimental Immunology*, 73: 449–455) with permission.

cultures used at the time were different from most of those previously described and the majority described since. It had been customary to culture the adherent cells derived from synovium (synoviocytes) in serial passage, resulting in a population of fibroblast-like cells. While the properties of these fibroblast-like cells which have been described variously as 'activated' or 'transformed', they by no means represented the totality of the rheumatoid synovium where the great majority of the cells are infiltrating leukocytes from the blood, such as T lymphocytes, macrophages, dendritic cells and plasma cells. Hence, in our approach, we cultured all the cells. By necessity, the duration of cell culture was relatively short term, as by 7 days many of the blood-borne cells, deprived of their growth factors, had died.

This simple experiment, in which all the synovial cells that were extracted by collagenase and DNAase digestion and cultured in vitro, without extrinsic stimulation, revealed that cytokine gene regulation in synovium was unusually prolonged. Most importantly it provided an in vitro model system for exploring the details of cytokine gene expression in a disease context.²⁰

This in vitro model was most elegantly used by Fionula Brennan²¹ to demonstrate that in rheumatoid (Figure 3), but not osteoarthritic synovial cultures without extrinsic stimulation, blockade of TNF by a specific antiserum was sufficient to abrogate IL-1 production within 3 days. Other control anti-sera, to lymphotoxin, for example, had no effect. This finding stimulated a series of experiments to investigate the effects of blocking TNF on multiple other pro-inflammatory cytokines (e.g. GM-CSF²², IL-6, IL-8²³). These led to the concept of the TNF-dependent cytokine cascade in which TNF is a prime

**ANALYSIS OF CYTOKINE REGULATION
REVEALED IMPORTANCE OF TUMOUR NECROSIS FACTOR**

APPROACH	Operative sample synovium, active RA cells isolated, placed in 'tissue culture'
OBSERVATION	Spontaneous production of many mediators of disease -cytokines, enzymes etc.
EXPERIMENT	Antibody to TNF inhibits production of other pro-inflammatory cytokines

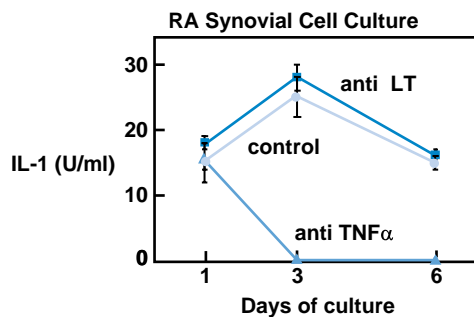


Figure 3. Blockade of TNF in synovial cell cultures inhibits IL-1 production, pointing to an important regulatory role for TNF in RA. Reproduced from Brennan FM et al (1989, *Lancet* ii: 244–247) with permission.

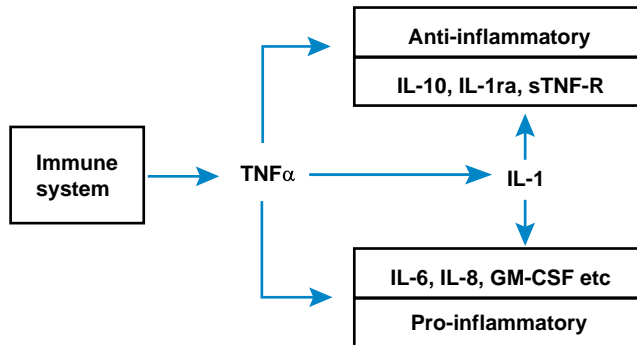


Figure 4. Cytokine cascade in RA.

mover in co-ordinating a cytokine response. Needless to say, like most other concepts in biology (Figure 4), is an over-simplification, but it has helped our thinking and progress towards a clinical evaluation of our hypothesis.^{24,25}

A number of other facts seemed to support the concept of TNF as a 'pivotal' cytokine, as distinct from other pro-inflammatory cytokines. Thus, after stress, TNF is the first cytokine to be detected in the blood (within 30 minutes)²⁶ and appears to raise the 'fire alarm' that calls in the 'firefighters' (i.e. the leukocytes) via inducing expression of adhesion molecules and chemokines. Furthermore, in a model of a 'normal' immune response, i.e. the response to infection with Gram-negative bacteria, blocking TNF markedly reduced and delayed the production of IL-1 and IL-6, thus indicating that the TNF-dependent cytokine cascade is not only confined to pathological tissues in disease but a part of a homeostatic physiological response.²⁶

In situ expression of cytokines in synovium

While a detailed analysis of cytokine regulation in rheumatoid synovial cultures suggested the importance of TNF in the pathogenesis of RA, there remained a major potential problem. TNF is very rapidly induced by extrinsic stimuli, including lipopolysaccharides (LPS), and so it seemed possible that in vitro production of TNF may be an artifact and does not reflect detection of a molecule capable of mediating in vivo pathology. Critical confirmatory evidence of its in vivo importance came from in situ expression of cytokines from tissues obtained and frozen quickly, before TNF could be induced in vitro. A number of groups revealed up-regulated expression of TNF and TNF receptors in synovium^{16,27,28}, thus supporting the concept that TNF might be a therapeutic target.

Animal models of RA respond to TNF blockade

While local in situ expression data and in vitro RA synovial regulation data provide evidence that TNF might be a therapeutic target, the response of animal models of RA to monoclonal anti-TNF antibody provided further supporting evidence. However, animal models of disease are notoriously poor predictors of the response of human disease, as evidenced by multiple publications. Many drugs are effective in animal models but not in humans.²⁹ Thus, anti-CD4 antibody proved to be very effective in suppressing

collagen-induced arthritis but not when applied in patients with RA. One key difference between the animal experiments and the human clinical trials was that, in mice, anti-CD4 was administered at the time of immunization as preventative therapy, i.e. well before the onset of disease.⁴ We therefore decided to test the effects of TNF blockade in established collagen-induced arthritis. Collagen-induced arthritis is a T- and B-cell-dependent polyarthritis that occurs in genetically susceptible strains of mice and rats following immunization with type II collagen emulsified in complete Freund's adjuvant.³⁰ The histological features of collagen-induced arthritis closely resemble those of human RA, including the localization of erosions in the synovial/bone/cartilage junction. Monoclonal hamster anti-TNF- α antibody (generously donated by Bob Schreiber³¹) was used to validate the hypothesis that TNF- α blockade would be effective in reducing the severity of established collagen-induced arthritis. It was found that anti-TNF- α treatment reduced clinical disease severity in a dose-dependent manner, with 50 μg twice/week being ineffective but 300 or 500 μg twice/week controlling clinical severity.³² Next, a histological analysis of the arthritic joints of treated mice was carried out and this revealed that there was considerably less cellular infiltrate in the treated joints, and less destruction of the joint architecture. In addition, there was less evidence of apoptosis of chondrocytes in mice treated with anti-TNF- α , less roughening of the cartilage surface and less erosions at the cartilage/bone/synovium junction. Anti-TNF was also found to act synergistically with anti-CD4 in suppressing both clinical and histological parameters of disease severity³³ (Figure 5).

In addition to our own findings, Thorbecke et al and Piguet et al^{34,35}, performed similar studies and both demonstrated beneficial effects of TNF- α blockade in collagen-induced arthritis. The results were also consistent with findings from other models of arthritis and also with the observation that over-expression of huTNF- α in transgenic mice results in spontaneous onset of arthritis that can be prevented by administration of anti-human anti-TNF- α monoclonal antibodies (mAb).³⁶

Thus, a number of strands of evidence pointed to TNF as a potential therapeutic target; the rationale for anti-TNF- α therapy in RA

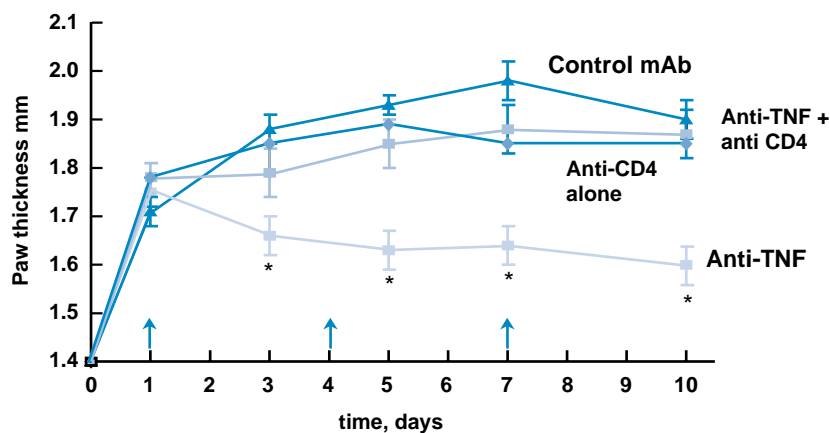


Figure 5. Combination therapy: suboptimal doses of anti-TNF exhibit a synergistic effect with anti-CD4 in the amelioration of collagen-induced arthritis. Anti-TNF (50 $\mu\text{g}/\text{mouse}$) and anti-CD4 (200 $\mu\text{g}/\text{mouse}$) were given by intraperitoneal injection every third day. Treatment was started after onset of arthritis. Reproduced from Williams RO et al (1994, *Proceedings of the National Academy of Sciences of the USA*, 91: 2762–2766) with permission. Copyright © 1994, National Academy of Sciences, USA.

- Dysregulated cytokine network in RA synovium is dependent on TNF- α .
- TNF- α /TNF-receptor is upregulated in synovium.
- Animal model of RA responds very well to anti-TNF- α administered after onset of disease.

THERAPEUTIC AGENTS WHICH BLOCK TNF

The work of Bruce Beutler, Tony Cerami and their colleagues^{37,38} in the 1980s showed that TNF had a major role in the pathogenesis of the sepsis syndrome in response to Gram-negative bacteria. This was convincingly shown in a variety of species, and models, and led to a number of clinical trials to evaluate this hypothesis in human sepsis. A variety of therapeutic agents were prepared by various companies in order to generate 'blockbuster' drugs for this seemingly lucrative indication with a clearly unmet medical need. Thus: Roche prepared a p55 TNF receptor human IgG1 Fc fusion protein, lenercept. Immunex prepared a p75 TNF receptor human IgG1 Fc fusion protein, etanercept (Figure 6). Centocor prepared a chimeric (mouse Fc, human IgG1 Fc) mAb and Celltech prepared a humanized mAb CDP571. Thus, appropriate therapeutic tools which had been produced and tested in humans in the attempt to treat sepsis were available to test our hypothesis.

However, it is not a trivial problem to convince companies with suitable tools to alter their pre-clinical use for a new indication instead. This is because of the costs of clinical trials, and the problems that would ensue, affect the entire development programme with a therapeutic if unacceptable toxicities are documented in the course of the trials for an entirely novel indication. Due to these concerns we had problems convincing the first company we approached of the strength of the rationale for TNF blockade in RA.

The second company we propositioned, Centocor, was convinced that there was a strong rationale, and that the safety aspects were not insurmountable. As usual, this decision depended on the insight of a single scientist, with appropriate medical and scientific knowledge, Jim Woody, at the time chief scientist at Centocor.

CLINICAL TRIALS

The chimeric antibody to TNF developed by Centocor from a murine hybridoma, A2, generated in Jan Vilcek's laboratory by J. Lee, was termed cA2 (chimeric A2). It was chimerized by David Knight and John Ghrayeb³⁹ by grafting the murine Fv segments to a human IgG1,K backbone, and produced in large quantities sufficient for clinical trials; phase I studies had already been performed before rheumatoid studies were initiated.

A feature of the therapy of murine collagen-induced arthritis with Bob Schreiber's hamster anti-murine TNF antibody was the marked dose-response. Two doses per week of 300 μ g (10–12 mg/kg) stabilized the inflammation, whereas 50 μ g (~2 mg/kg) was ineffective.³² With the high affinity of the assembled trimeric TNF receptor for trimeric TNF, it is perhaps not surprising that a marked excess of antibody was needed.

With a novel therapeutic concept, with no proof of its veracity, the initial trial could be the last. Hence, we were very careful to design a trial with the maximum probability of success, within the ethical boundaries. This involved treating patients who had failed all available therapy and still had active disease. To maximize the chance of success

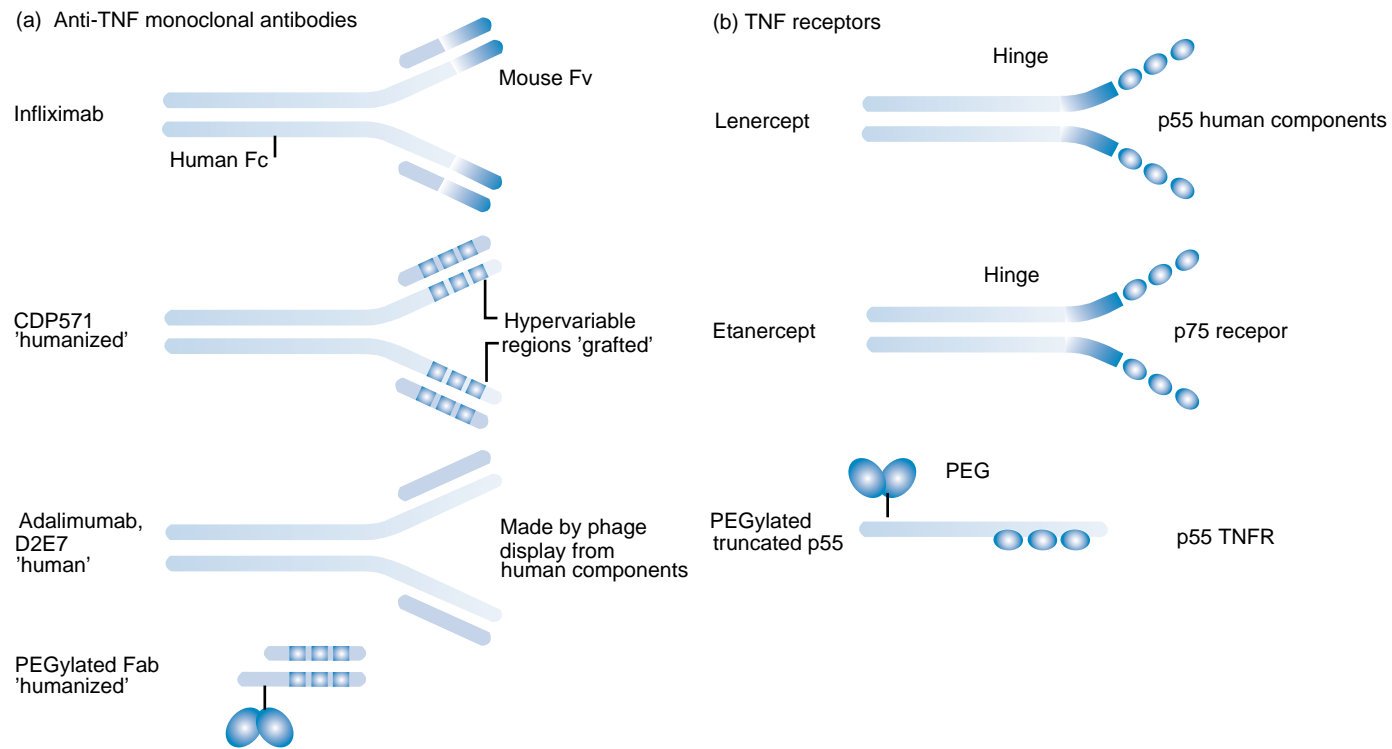


Figure 6. Diversity of therapeutic agents developed by various companies to inhibit TNF activity in patients. Reprinted by permission from *Nature Reviews Immunology* (vol. 2, pp. 364–371) © 2002 Macmillan Magazines Ltd (www.nature.com/reviews).

the highest dose possible (20 mg/kg over a 2-week period) was used. This dose was obtained from prior toxicology studies. As the results and safety were unpredictable, the burden of a double-blind randomized protocol was not appropriate.

The results were as good as we could have hoped for, perhaps even better.⁴⁰ There was strong symptomatic response, with relief of tiredness and lethargy, relief of morning stiffness, diminution of pain (Figure 7), and by clinical examination, reduction of joint swelling and tenderness. But the most telling results came from blood tests which demonstrated that CRP, ESR and IL-6 were markedly reduced within a week of initiating therapy (as was rheumatoid factor after 8 weeks), verifying objectively what had been subjectively indicated by changes in symptoms and signs.

The emotional responses generated during such a clinical trial can only be guessed at by those who did not take part—the patients and investigators experienced joy at the prospect of major benefit, but as the benefit wore off and they relapsed, gloom and depression followed. The anxiety that repeated therapy may not work was relieved when further cycles of the anti-TNF antibody were associated with a similar therapeutic response, albeit in some patients the duration of benefit showed some reduction.⁴¹

FORMAL PROOF OF PRINCIPLE

While the first open trial of 20 patients was performed solely at Charing Cross Hospital⁴⁰, the formal proof of principle, a double-blind randomized placebo-controlled trial was performed in four European centres, with the assistance of the teams of Ferry Breedveld in Leiden, Joachim Kalden in Erlangen and Joseph Smolen in Vienna. This was a simple trial design in which two doses of cA2 (1 and 10 mg/kg) were compared against a placebo. So that its appearance was the same, of a protein solution, human serum albumin was used, as it was considered unethical to infuse a potentially immunogenic control chimeric antibody. To ensure that the therapeutic effect noted was due to anti-TNF (Figure 8), the patients were ‘washed out’ of existing therapeutic agents for at least 4 weeks.⁴²

In an attempt to ensure that placebo patients did not drop out of the trial, with the ensuing loss of statistical power, the primary end-point of the trial, a composite measure of clinical response was recorded at 4 weeks, shorter than the optimal time point for effects seen in the open trial. The therapeutic response, measured by a validated criteria developed by Harold Paulus⁴³ was very clear cut 7% of placebo-treated patients met the criteria compared to 44% at 1 mg/kg cA2 ($P = 0.0083$) and 79% of 10 mg/kg cA2 ($P = < 0.0001$).⁴²

DOES TNF BLOCKADE HAVE LONG-TERM EFFICACY? LONGER-TERM CLINICAL TRIALS

While the 4-week randomized double-blind placebo-controlled trials constitute the formal proof of principle, in terms of the lifespan of a patient with RA it is a benefit of little consequence because all patients showed a relapse of their disease. The key issue was whether patients could be re-treated, whether cA2 would be too immunogenic, and whether, in the absence of TNF, another pro-inflammatory cytokine would step in to drive the disease activity.

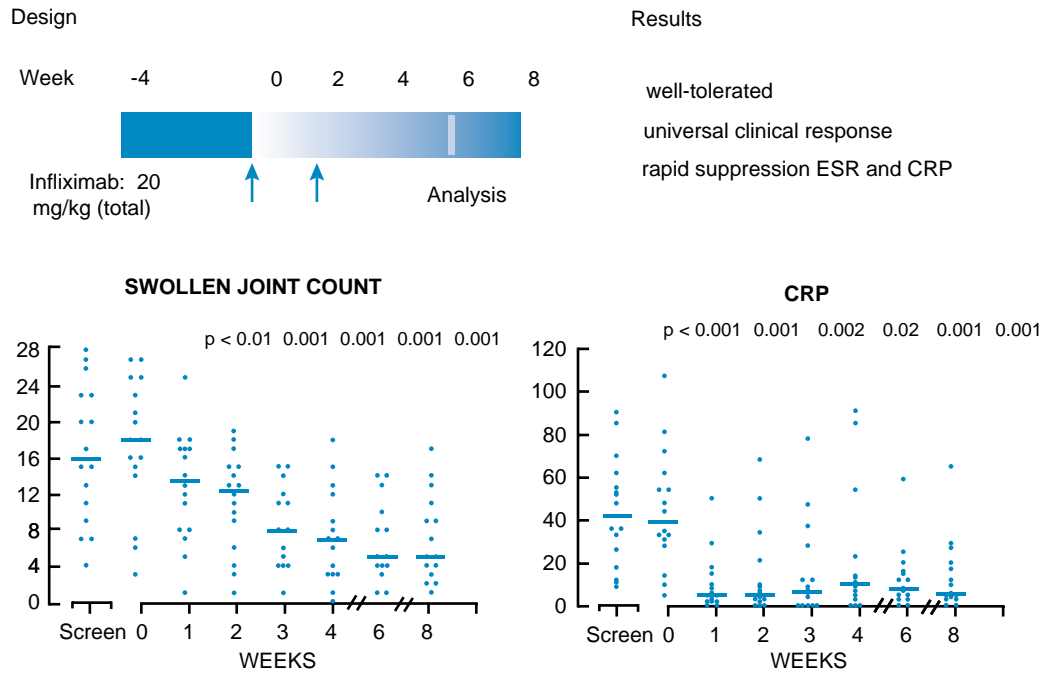
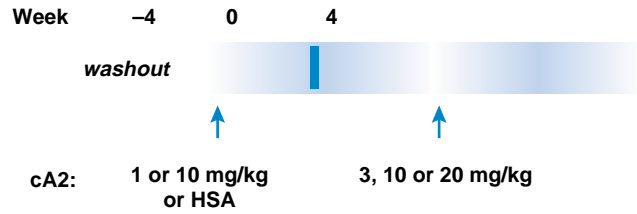


Figure 7. Open-label treatment with infliximab in RA. Reproduced from Elliott MJ et al (1993, *Arthritis and Rheumatism* 36: 1681–1690) with permission.

Design



Results

well tolerated
good clinical responses
in cA2 groups
dose-response
relationship

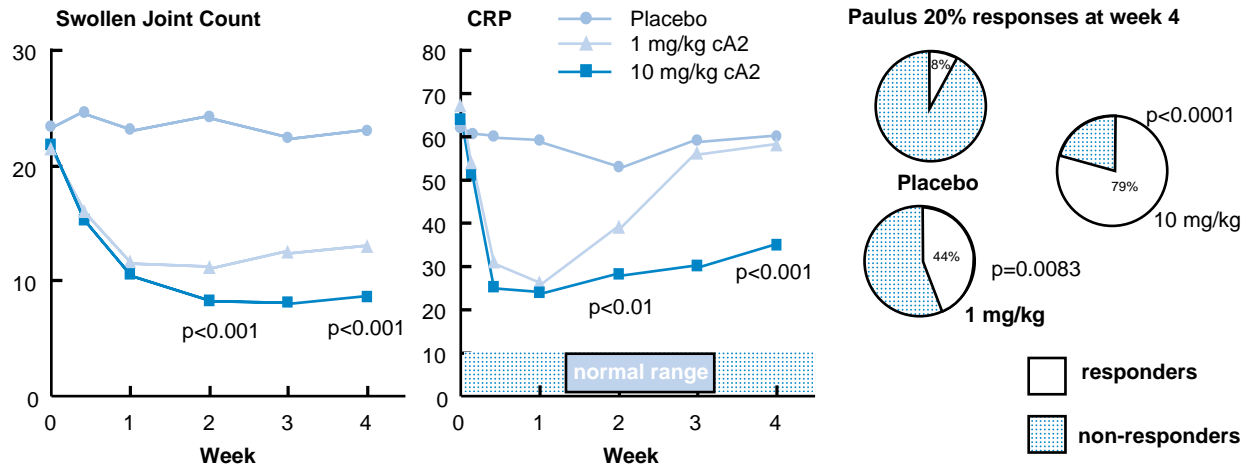


Figure 8. Randomized, placebo-controlled trial of infliximab in RA. Reproduced from Elliott MJ et al (1994, *Lancet* 344: 1105–1110) with permission.

The first clue that long-term benefit might be possible came from the re-treatment of eight patients of our first open study, in which benefit was re-induced on three or four occasions after relapse.⁴¹

A more formal evaluation of longer-term benefit was performed in patients with an inadequate response to methotrexate (MTX). A dose response of 1, 3 and 10 mg/kg of cA2 without or with MTX, at a low fixed dose of 7.5 mg/week, was compared with methotrexate alone as the control. Five infusions were administered in each group over 13 weeks, with the final assessment 14 weeks later.⁴⁴ This phase II trial has been very influential in determining the routine use of infliximab (Figure 9). It was found that, in the absence of MTX, 1 mg/kg was not effective long term, 3 and 10 mg/kg were effective in the absence of MTX, but that all doses were more effective with MTX. The mechanism of the MTX synergistic action is not fully understood—higher blood levels were detected, and these were much reduced levels of anti-idiotypic antibody response.⁴⁵ This led to the phase III trial being performed in the presence of MTX, and the FDA/EMA approved the use of infliximab in RA in combination with MTX, as described below.

The phase III randomized controlled trial of infliximab plus MTX (the ATTRACT trial) compared four dosing schedules—3 or 10 mg/kg every 4 and 8 weeks versus MTX and placebo infusions. Observations at 30, 54 and 102 weeks showed a sustained improvement in signs and symptoms in ~50% of patients, attenuation or infiltration of progression and possible healing of bone erosions and joint space narrowing (cartilage damage) in a majority of patients and an improvement in physical function, vitality and social functioning. These data, in a population of RA patients with moderate to severe disease activity despite prior treatment with multiple disease modifying anti rheumatic drugs (DMARDs), translate into convincing evidence for anti-TNF therapy in such patients. The introduction of infliximab into clinical practice has proved its effectiveness with retention rates of 60–70% on treatment for the 2–3 years since its availability as a licensed product.^{46,47}

A role for TNF in protective immunity against infections has been established in experimental models. Hence, an increased risk of infections arising from anti-TNF therapy was anticipated. In clinical trials, the incidence of infections—such as sinusitis and infections requiring antibiotics—was marginally increased but serious infections did not occur with increased frequency compared to MTX-treated controls. Post-marketing reports of tuberculosis, histoplasmosis, listeriosis, coccidiomycosis, pneumocytis and bacterial infections leave little doubt of the small but increased risk of certain infections. Exclusion of susceptible populations, screening for infections and eradication prior to therapy, and close monitoring of patients under treatment, allow adequate management of the risk of infection.^{46–50}

Other safety issues include the rare occurrence of drug-induced lupus (reversible on cessation of therapy)^{51,52}, possible aggravation and induction of a demyelinating syndrome, rare cases of anaphylaxis or anaphylactoid reactions, and unexpected worsening and/or death of patients with congestive cardiac failure.⁵³ The reported cases of lymphoma exceed that observed in the general population. Because patients with long-standing and persistently active RA—a population analogous to that selected for anti-TNF therapy—have an increased risk of lymphoma, it is uncertain as yet whether anti-TNF therapy contributes to the evolution of this malignancy.⁵⁴ However, there is no sign of an increase in other malignancies.⁵⁵

On current evidence it may be concluded that the benefits of anti-TNF outweigh risks in the patient population for whom this therapeutic option is indicated.^{46,49}

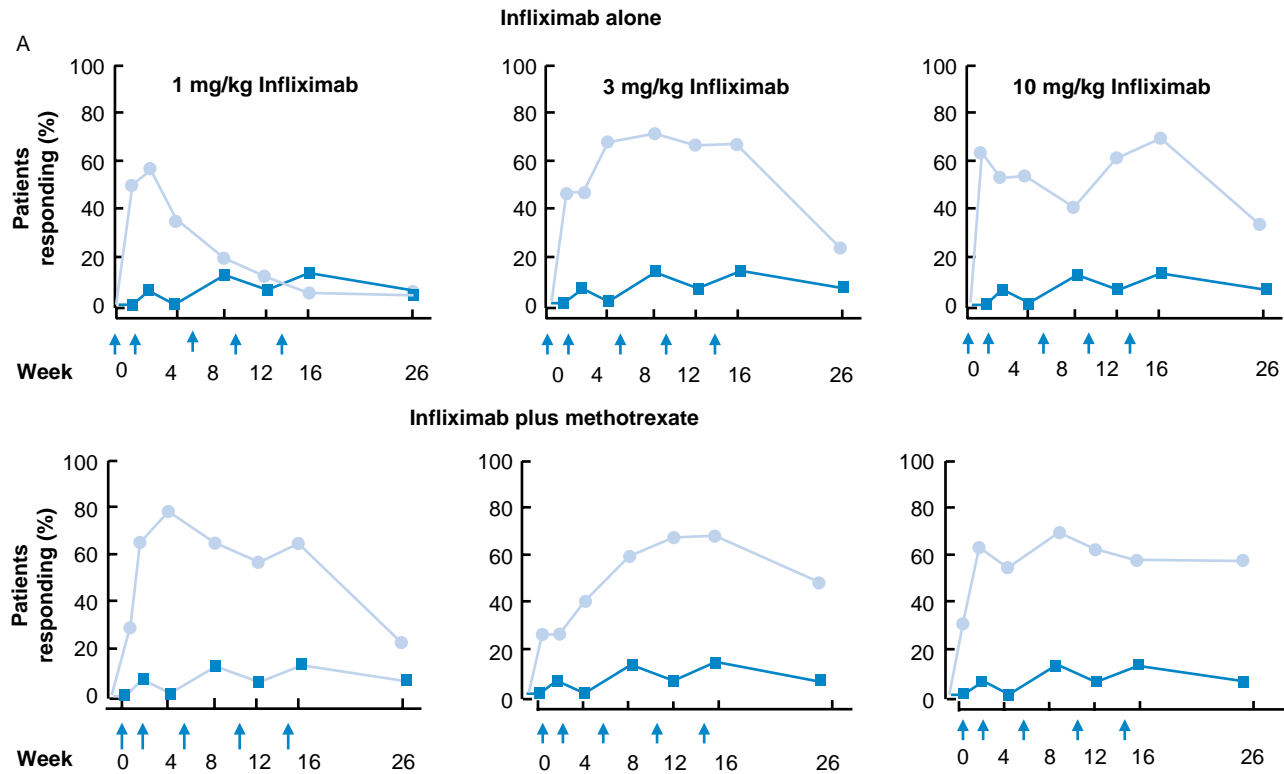


Figure 9. (A) Infliximab monotherapy versus combination therapy RCT. (B) Combined treatment with infliximab and methotrexate inhibits the antibody response to infliximab and leads to increased levels of infliximab in the circulation. Reproduced from Maini RN et al (1998, *Arthritis and Rheumatism* 41: 1552–1563) with permission.

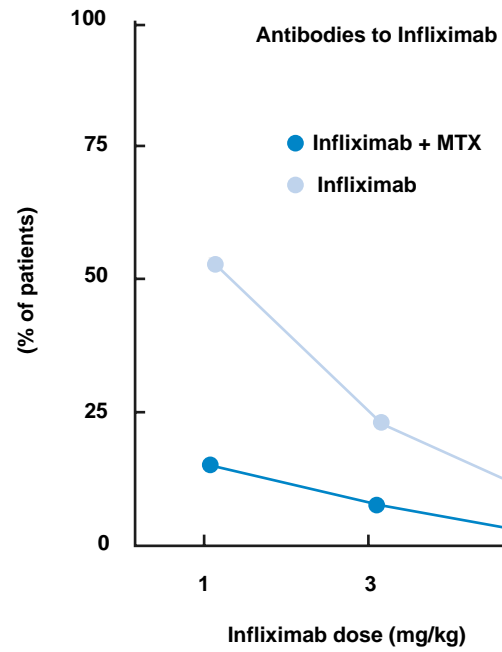
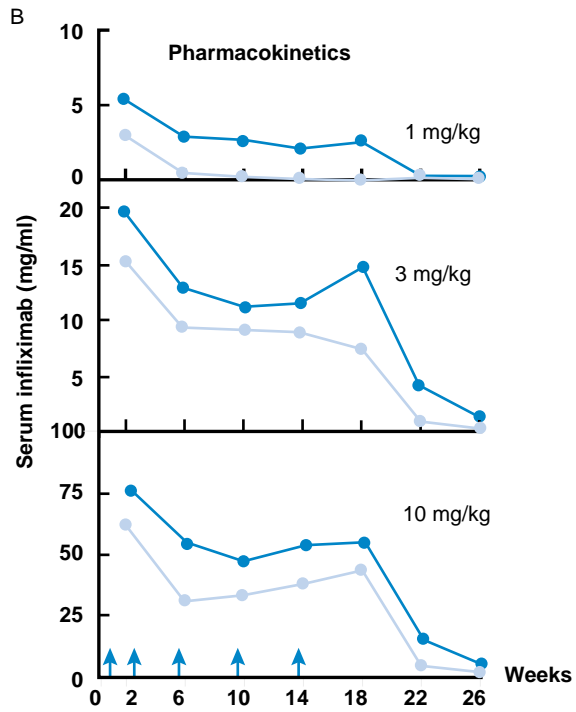


Figure 9

COMPETITION AND EARLY DISCLOSURE IS GOOD FOR PATIENTS

A principle of science is early disclosure of important information, and this was the case here. The results of the open trials were disclosed within a few months of the trial beginning (in May, 1992), at a meeting in Arad, Israel, in October 1992. With representatives of other companies possessing anti-TNF therapeutics present at Arad, the stage was set for having other groups verify the hypothesis that TNF blockade was therapeutic in RA. The first confirmatory report came from Celltech⁵⁶, using a humanized mAb, then from Roche⁵⁷ and Immunex with TNF receptor-based fusion proteins.

However, it is noteworthy that not all of these therapeutics which confirmed the principle have become commercial successes. Immunex's (now Amgen/Wyeth's) etanercept selling as 'Enbrel' is a commercial success after its rapid development^{58,59}, whereas the other two stalled during the complex and costly development process. But commercial competition is very good for the patients because it provides choice, financial competition and, most importantly, diversity of supply of products.

MECHANISM OF ACTION STUDIES

The clinical studies with infliximab (Remicade[®]), the anti-TNF now sold by Centocor/J&J/Schering-Plough, showed marked clinical benefit.^{40,41,44} This provided a relatively unique opportunity to study the mechanism of benefit of a drug with a relatively defined biochemical mode of action. This would provide information about the pathogenesis of the disease process, as revealed by the concomitant changes during benefit and relapses.

The studies performed during the analysis of TNF blockade were more extensive than usual; this is probably (in our limited experience) because the development of TNF blockade started as an academic–industrial collaboration and not as a routine pharmaceutical development. The former provides many more opportunities for research, whereas in routine pharmaceutical development clinical research may often be seen as a distraction which delays clinical trials and detracts from the critical path towards drug approval (Figure 10).

There were a number of studies performed to evaluate whether the mechanisms observed *in vitro* were operative *in vivo*. The 'cytokine cascade' operated *in vivo*; thus, anti-TNF-induced reductions in levels of IL-6 were noted⁶⁰—as were reductions in IL-1⁶¹, VEGF⁶², IL-8 and other chemokines.⁶³

Immunohistological studies were performed to investigate the changes in the synovium; reductions in the expression of adhesion molecules and reduction in cellularity, etc. were observed.⁶⁴ Reductions in angiogenic factors and angiogenesis were also noted.^{62,65}

A very important study, which we think explains why TNF blockade is useful in very many diseases, was published by Taylor et al.⁶³ It used radiolabelled granulocytes to demonstrate that the influx of labelled granulocytes was reduced by about 50% in the joints 2 weeks after a single dose of infliximab. As other leukocytes share chemokines and adhesion molecules with granulocytes this study indicates that reduced recruitment of leukocytes to joints is an important aspect of the benefit of anti-TNF therapy.

An aspect which has never been clarified is whether IgG1 mAb such as infliximab are capable of lysing TNF-expressing cells (e.g. macrophages) *in vivo*.⁶⁶ *In vitro*, it is

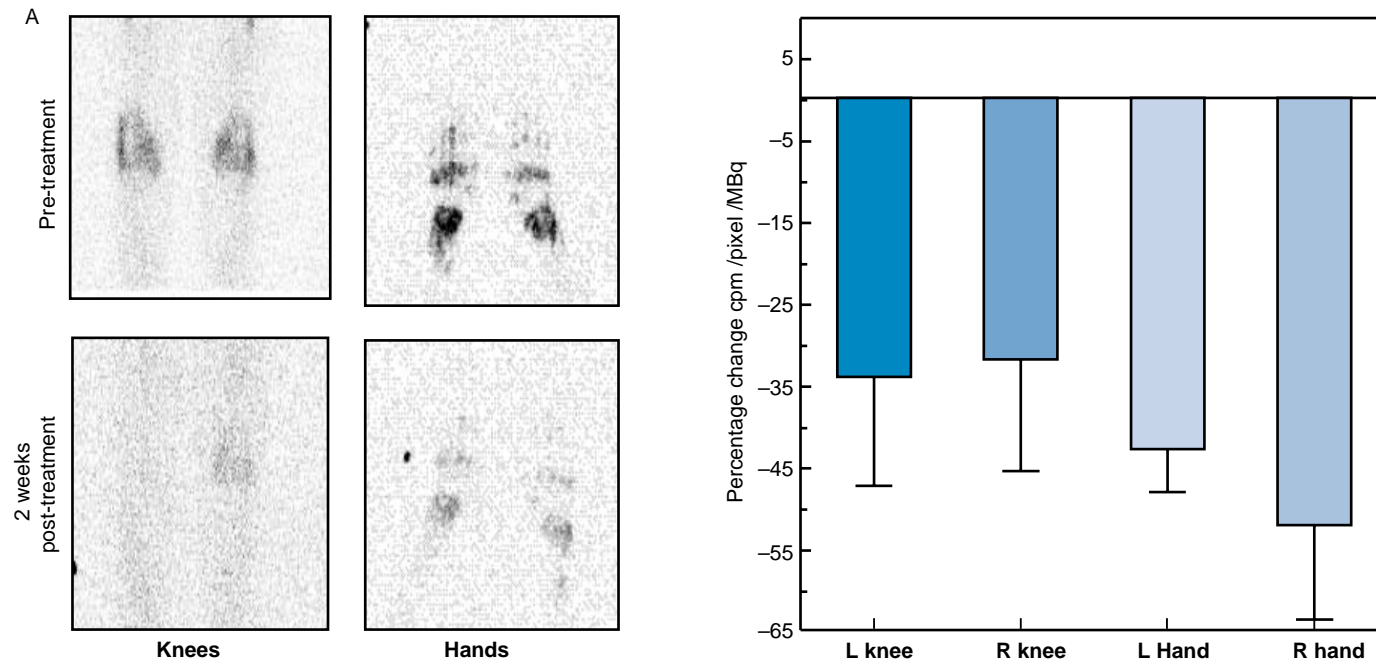


Figure 10. (A) Treatment of RA patients with infliximab significantly reduces ^{111}In Indium-labelled granulocyte migration into affected joints. Reproduced from Taylor PC et al (2000, *Arthritis and Rheumatism* 43: 38–47) with permission. (B) Treatment of RA patients with infliximab reduces circulating levels of VEGF and the level of vascularity of the synovium. Left-hand panel reproduced from Paleolog EM et al (1998, *Arthritis and Rheumatism* 41: 1258–1265); right-hand panel reproduced from Taylor PC et al (1998, *Arthritis and Rheumatism* 41: S295) with permission. © 1998, 2000. Reprinted by permission of Wiley–Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

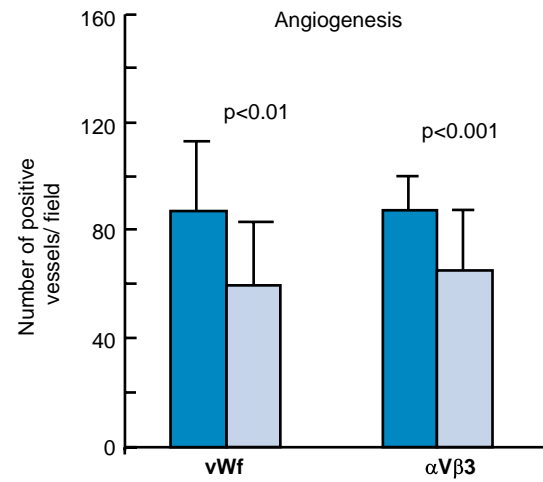
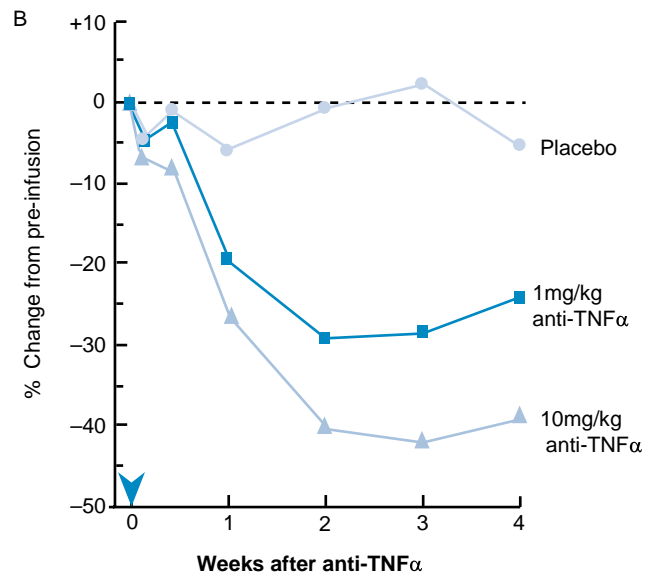


Figure 10 (continued)

possible, but with target cells that had non-sheddable TNF, with higher levels of infliximab than are reached in vitro, and with non-human complement.

LESSONS YET TO LEARN

Some of the unexpected adverse events observed with the use of anti-TNF agents have yet to be explained. While the increased susceptibility to infections was anticipated the induction of dsDNA antibodies in about 15% was expected on the basis of animal models⁶⁷, and rare cases of lupus need to be explored further.⁵¹ Similarly, the rare cases of demyelinating syndrome that occur is another as yet unexplained clinical finding.⁶⁸ That both syndromes largely resolve when therapy is discontinued argues for the brief interruption of a controlling system. It is not often that such clear clinical clues make themselves apparent.

WHAT HAVE WE LEARNT ABOUT 'TRANSLATIONAL MEDICINE', THE TRANSITION OF CONCEPTS FROM LABORATORY TO CLINIC?

In summary, it is a complex and uncertain process, involving industry in a multi-million dollar gamble, or risk.

1. A well founded rationale is essential, but insufficient. This is obvious for many reasons, but most importantly what seems a strong rationale to academic experts may not be to industry. There may be no experts in the company, so they need to get consultants whose knowledge may or may not be commensurate with the task. For industry the best founded rationale is an existing proof of principle trial. In the example of TNF blockade, this was the situation for Immunex and Abbott.
2. Patents are important, but not essential. Patents enable the owner to prevent (often at high expense) someone else infringing his rights. They do not necessarily provide the ability to sell a commercial product, which might infringe the rights of others. The ability to sell is termed 'freedom to operate'. As many patents may be infringed by any new product, if the rights need to be purchased, the costs add up. This may lead to certain products being too risky commercially.

With patent coverage, the probability of commercial development is higher as the capacity to block others from competing reduces the risk of investment into a new project. So the academic institutions who wish to promote the development of their ideas strongly favour patenting new ideas with therapeutic potential.

3. Market size is important, but is usually not known. The risks of pharmaceutical development are well known. Only a small fraction of pre-clinical projects reach patients. Only a fraction of clinical projects reach patients. Hence the potential return on investment is a critical factor in whether the risk is justified. And risk there is: if a project product fails badly, jobs are involved, of the people who supported the project are at risk. There is little personal hazard in refusing to take on a potentially risky new concept.

The problem here is that while markets with existing products can be modelled with a degree of insight, with no existing market the guesses vary considerably. Before cyclosporin there was essentially no market for immunosuppressive drugs, and Sandoz thus virtually abandoned it.

Market size and patient numbers do not necessarily tally. Thus, Genzyme has sold a lot of enzyme for saving the life of a few patients with Gaucher's disease. The pricing of drugs is far removed from cost-of-goods as it has to factor in the research costs, clinical trials, regulatory affairs, marketing, licensing and insurance, as well as covering the costs of the projects which failed along the way. Hence, the strenuous efforts of pharmaceutical companies to get into markets that have already been defined; for example in the anti-ulcer market, the later entries—with some improvements over the innovator—have, made more money. In terms of anti-TNF therapy, it is clear that the market sizes are large, due to the complexity and cost of treatment with biologicals.

4. Competition is good for both patients and industry. The interest of multiple companies in a single therapeutic area may seem to be good for the patients as it provides choice and improvements, but perhaps not for the pharmaceutical companies. However, with anti-TNF it is clear that the market can support at least three large competitors. In fact the same is true for many therapeutic areas, such as hypertension, high cholesterol, etc.

The competition between infliximab and etanercept, visible in TV advertisements in the USA, raises awareness from patients and is less likely to lead to under-treatment, as in Europe.

5. Drugs with multiple uses are very much appreciated. The cheapest drug to development is a new indication for an existing drug. All the toxicity/safety/pharmacology, etc. is already done. Regulatory approval is also more rapid.

Anti-TNF biologicals certainly fit into this category, being currently approved for adult RA, juvenile RA, Crohn's disease, ankylosing spondylitis and psoriatic arthritis, with many more indications under investigation with positive anecdotal reports.

6. Clinical trial design needs to match the unmet medical need. In the 1990s the utility of methotrexate in RA, championed by Michael Weinblatt, led to a revolution. MTX became the gold standard. So the unmet need was MTX non-responders or toxicity—and thus infliximab trials targeted this population. Now there is a need for therapies that can be safely used in the 20–40% of patients not responding to TNF inhibitors.
7. Most important, there has to be an atmosphere of trust between laboratory workers and clinicians, if the academic–pharmaceutical partnership is to work.
8. Spawning of new industries. The publication of the 'proof of principle' trial with cA2 described earlier⁴⁰ stimulated the launch of a large number of pharmaceutical and academic programme to find targets in the TNF cascade that would be 'druggable'. There was a strong sense of urgency, as the data were compelling. Virtually ever major pharmaceutical company and many Biotech companies either resurrected, began or changed the direction of other programmes to find orally active TNF inhibitors. Ten years later a few companies are beginning the testing of compounds in phase I-II clinical trials. Among others, Boehringer Ingelheim, Roche, RW Johnson, Scios, Tularik, Vertex, etc. are reported to have made inhibitors that block at various stages in the TNF production pathway. That a decade has passed is evidence of the difficulty of the task, even when the certainty of a hugely successful product is clear. Given current trial length times, the need to now show evidence of joint protection, and the increasing regulatory hurdles, it may be another 3–5 years before any of these emerge as therapeutics.

SUMMARY

The application of laboratory knowledge and its conversion into practical therapeutics is a fraught, long and expensive process, with a horrendous failure rate and monumental

costs. We describe here some of the steps in translation of laboratory knowledge into clinical therapeutics, and review some of the things that we have learnt.

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