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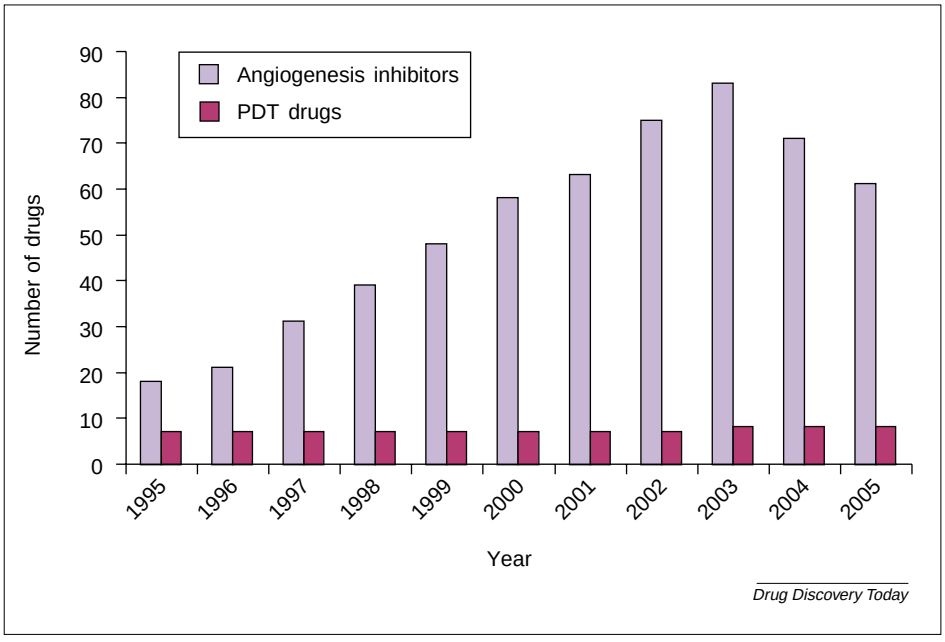

FIGURE 3

Comparison of angiogenesis inhibitors and photodynamic therapy drug development over the past 10 years. The development rate of angiogenesis inhibitors for the treatment of ophthalmological conditions and cancer has exceeded the rate in development of photodynamic drugs for the same indications sixfold between 1995 and 2003.

US-based Genaera has recognized the importance of angiogenesis inhibitors as significant future therapies and has taken the strategic decision to focus the majority of its resources on its Phase II compound, squalamine, an antibiotic discovered in dogfish shark tissue for the treatment of wet AMD and cancer.

Encouraging outlook

Pharmaprojects has revealed a significant number of drugs sharing a pharmacological activity that are under development for both cancer and wet AMD. The success of pegaptanib octasodium and bevacizumab as angiogenesis inhibitors (launched for the treatment of wet AMD and cancer, respectively, and in development for cancer and wet AMD, respectively), indicates the tremendous potential that antiangiogenic therapy will have in the future. With a significant proportion of the drugs highlighted by Pharmaprojects also focusing on blocking various stages of angiogenesis rather than solely disrupting the function of VEGF (as with pegaptanib octasodium and bevacizumab), the outlook for future treatments remains positive.

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Broad spectrum immune monitoring in immune-mediated inflammatory disorders

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The high attrition rate of new candidate drugs is a major concern for the drug development industry and an intensively discussed subject in this journal. The translation of data from preclinical research in relatively simple laboratory animal models to the complex diseases in the human population appears to be the Achilles heel of the drug development process. Multiplexed assay systems could help to better understand the crucial differences between pathogenic processes in animal models and the human disease.

Introduction

The ageing societies of Europe and North America are facing an increasing prevalence of chronic immune-mediated inflammatory disorders (IMID), such as rheumatoid arthritis (RA), multiple sclerosis (MS) and inflammatory bowel disease (IBD). During the past decades the drug development industry has invested heavily in biotechnology research aiming to identify the most relevant therapeutic targets for these conditions and to develop safe, effective therapies for them. Despite years of intensive research in industry and academia, really effective therapies are lacking for the majority of IMID. Moreover, several clinical

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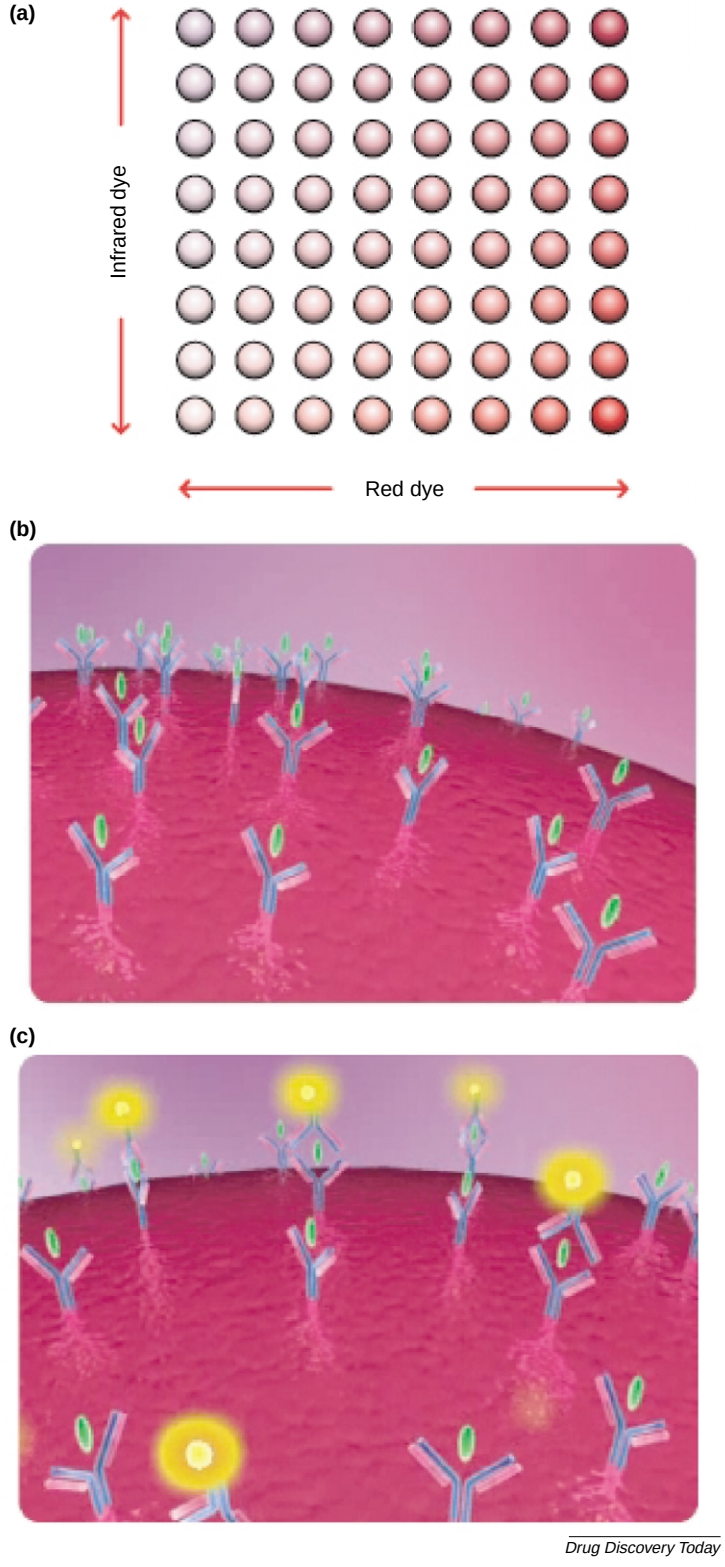

FIGURE 1

The principle of x-MAP multiplex technology. Microsphere-based flow cytometry combines the principles of flow cytometry with a solid-phase assay (e.g. ELISA). The technique developed by Luminex uses sets of polystyrene microspheres that are filled with red and infrared dyes at different ratios **(a)**. Coated on the surface of each microsphere subset is a biological probe for the detection of a specific analyte **(b)**, which can be a monoclonal antibody, a receptor, an antigen, an oligonucleotide molecule, among others. In a typical multiplex analysis of a biological sample, mixtures of up to 100 different dye-labeled microspheres are incubated and subsequently developed with a specific detection reagent for the analyte **(c)**, for example a fluorochrome-labeled monoclonal antibody. After development, the bead mixture will be analyzed by flow cytometry, monitoring the amount of fluorescence on each microsphere subset as a function of the analyte concentration.

trials of new therapeutics had to be stopped because they lacked activity or were even detrimental. The poor predictive value of the current, preclinical animal models is clearly the Achilles heel of the drug development process, a fact that has been addressed in previous publications of this journal [1,2].

A complicating factor in therapy development is our poor understanding of the complexity of pathogenic processes [3,4]. For example, the concept that MS is caused by autoreactive T helper 1 cells attacking the myelin sheaths that wrap around the axons of the central nervous system (CNS) has prevailed for a long time [5]. More-recent concepts show that MS actually represents a group of disorders with heterogeneous clinical and neuropathological presentations. Complex networks of different biological systems interact in the immunopathogenesis of MS, including cytokines, chemokines, complement factors, hormones from the hypothalamus–pituitary–adrenal axis and sex hormones, among others [6–10].

Thus, it can be envisaged that factors within single or different networks might change in tandem, in the same or opposite direction. Interactions might vary with time and differ between pathological situations. For example, antibody-mediated neutralization of TNF-α is a highly successful treatment for RA but it appears to be detrimental in MS [11]. By contrast, although interferon-β (IFN-β) is a registered treatment for MS it only has limited activity in RA [12] and might even promote autoimmunity in other conditions [13]. An example of a drug with opposite effects in the same disease is the glucocorticoid hormone dexamethasone. It is an effective inhibitor of MS-like disease in adult rats but it renders these animals more susceptible to

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TABLE 1

Comparison of microsphere-based flowcytometry with ELISA-based detection systems

	Traditional ELISA	Multi-spot test[a]	BD FACSArray™	Luminex xMAP® Technology
Instrument costs (US$)	<50 k	>100 k	50–100 k	50–100 k
Level of multiplexing	None	Up to 100	Up to 72	Up to 100
Sensitivity	Low pg/ml	Low pg/ml	Low pg/ml	Low pg/ml
Specificity	High	High	High	High
Internal controls	No	Yes	Yes	Yes
Liquid phase kinetics	No	No	Yes	Yes
Throughput (plates per day)	5–10	>10	5–10	5–10
Interassay reproducibility	<10% CV	>10% CV	<10% CV	<10% CV
Assay flexibility	Kits or homebrew	Kits only	Kits only	Kits or homebrew
Available application (kit menu)	Broad, many vendors	Small	Small, only from BD	Broad, many vendors
Application	Protein	Protein	Protein	Protein and nucleotides

[a]The multispot test is a modification of the traditional ELISA, among others used in HIV diagnosis [35].

the disease when administered early on in their life-cycle [14]. Such paradoxical data provide strategic information in the risk and efficacy assessments of new therapies for IMID. Therefore, we propose that broad-spectrum monitoring of these networks should be an integral part of preclinical and clinical validation experiments for new therapies.

From monoplex to multiplex measurement

The validity of drug tests in the current animal models for MS can be debated because the majority of new therapies capable of inhibiting disease in the experimental autoimmune encephalomyelitis (EAE) model are ineffective or even cause detrimental effects in patients [15]. Remarkably, the increasing awareness that the pathogenic mechanisms underpinning IMID in the human population are far more complex than those of the best current animal models has had only a minor impact on drug development strategies. How can this complexity be incorporated in the preclinical development of new therapies?

At a site of inflammation, many cytokines and chemokines are normally produced. The combined activity of such factors usually has a stronger impact on a cell or tissue than the individual factors themselves. Until a few years ago, it was impossible to determine the effect of a new therapeutic reagent, for example, an antibody against a single disease mediator on a complex pathogenic network. New multiplex bead-array techniques, with which a broad range of biological molecules can be simultaneously detected in a small sample volume, make this possible and practical (Figure 1). It does not require much imagination to see that these techniques will probably make an impact on the safety and efficacy profiling of new therapies.

Safety

One of the disease-modifying therapies used in MS is recombinant IFN-β. Interferons are endogenous, hormone-like proteins with strong antiviral, anti-tumoral and immunomodulatory properties [16]. A significant proportion of patients develop neutralizing antibodies that limit the efficacy of the treatment [17] and probably also neutralize IFN-β that is produced by their own bodies, for example in the response to (viral) infection. It is difficult to understand why the neutralization of such a crucial regulatory molecule in the immune system has no marked detrimental effects, as discussed in an earlier issue of *Drug Discovery Today* [17]. Is this as a result of compensatory mechanisms? If it is the case, could these compensatory mechanisms explain the limited effect of IFN-β in late-stage MS?

Efficacy

Inflammation is considered to be one of the key processes in MS and a major contributor to CNS pathology. Therefore, antibodies are being developed for the neutralization of important factors involved in the inflammation process, such as interleukin-12 and CD40 interacting with its ligand, CD154. Mouse models of EAE can be completely suppressed with such antibodies [18,19]. However, antibody treatment was only partially effective in a non-human primate model of the disease [20–22] but complete suppression of MRI-detectable inflammation in the brain had been achieved using anti-CD40 antibodies [23]. Such observations indicate that therapeutic antibodies could be less effective in more-complex animal models, which in turn are less complex than the human disease. It is clear that, where possible, preclinical test systems should take account of the high complexity of the human disease.

Multiplex assay systems

Microsphere-based flow cytometry is a relatively new technique for the simultaneous detection of a broad variety of molecules in a small sample volume [24] (Figure 1). The application can be diverse and includes detection by specific antibodies of cytokines [25] or peptide hormones [26], nuclear receptor–co-activator peptide-binding studies [27], single nucleotide polymorphism genotyping [28] and, as demonstrated recently, the study of microRNA expression profiles [29]. Compared with standard tests for detecting cytokines and antibodies these bead-based methods can be more accurate, more reproducible and require much less sample than ELISAs [30,31]. Table 1 compares some aspects of this new technology with more traditional assay systems. Current protocols are relatively low-to-medium density, detecting up to 100 analytes per sample. As the technology develops, it is expected that the number of parameters that can be simultaneously detected will increase rapidly [32,33].

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A typical preclinical therapy-validation experiment in non-human primates of a new therapeutic agent would utilize groups containing no fewer than five animals per group, which either receive the active molecule or a control substance. At periodic intervals, for example every week during a total monitoring period of 100 days, a blood sample will be taken that theoretically can be analyzed for up to 100 analytes. Assuming that all animals will reach the end of the observation period, the total dataset per experimental group will accumulate to 7500 data points. Clearly the extraction of information from such complex datasets requires advanced biometric techniques. A detailed discussion is beyond the scope of this feature publication. By way of illustration we refer to the analysis that we used in a 'fishing expedition' for biomarkers of CNS damage that could be detected with ^{1}H-NMR spectroscopy in the urine of MS patients, but not of healthy controls or other neurological diseases [34]. The described techniques can also be used to visualize changes in the pattern of analytes present in a body-fluid sample or culture supernatant sampled during the course of a pathological process or after administration of a therapeutic agent.

Conclusion

High-quality preclinical data facilitate the selection of the best candidate drugs from the development pipeline for clinical evaluation in IMID. The selected experimental models, as well as the chosen read-out parameters, have an impact on the validity of the preclinical data. It is particularly important that the chosen animal models and read-out parameters comply with the high complexity of most immune-mediated inflammatory disorders in the human population. More-complex animal models that are closely related to the respective human, clinical condition could be better at predicting the effect of a new therapy in patients. Moreover, multiplexed assay systems can help to assess possible adverse effects.

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