

# Methods and Findings

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# **Opening Conference**

## Therapeutic Innovation, Academy and Industry

*J.M. Palacios*

Agencia de Valorización y Comercialización de los Resultados de la Investigación (AVCRI), Barcelona, Spain.

At the beginning of the 21st century, humankind's state of health has experienced an impressive degree of improvement in comparison to the beginning of the 20th century. Not only do we live longer, we also live better, and in general we are healthier. Several factors have contributed to this state of well-being. One factor, and without a doubt a very significant one, has been the development of a great number of effective and safe medications. Yet, in spite of all this positive development, two-thirds of the nearly 30,000 identified diseases are still, to this day, not treated with an appropriate remedy. Moreover, the medical needs confronting such diseases remain urgent and very important.

In the last few years, the pharmaceutical industry, which has always been the principal engine for the development of new medicines, has been experiencing a significant decrease in its ability to generate new drugs. This is in spite of the revolution in knowledge that biology, chemistry and medicine have experienced in the last few decades. While investment in research and development in both the public and private realms of therapeutic innovation has undergone exponential increases (reaching US\$50 billion in the pharmaceutical industry in 2005), the number of new drugs approved still remains at record lows. In addition, the cost of developing new drugs continues to increase, now topping US\$800 million on the lower end to over US\$1 billion on the higher end.

Facing a stagnation in innovation, which has not only affected the pharmaceutical industry but also the public sector, university and public research organizations have reacted with proposals and plans to translate to society the fruits of research, in the form of new therapeutic interventions, in a quicker and more effective manner. The most significant proposals showing the most potential for greater impact are the NIH Roadmap of the National Institute of Health of the USA and the Critical Path to New Medical Products of the U.S. Food and Drug Administration (FDA), the American medicinal regulatory agency. In Europe, both the European Commission and many different organizations related to research and health have initiated similar projects.

All of these initiatives share common elements. One of them is the acknowledgement of the fact that public research cannot stop in the very initial stages of the drug-discovery process (i.e., at the identification of new therapeutic targets) but that the involvement should go much further beyond this point. The very process of creation of new therapeutic interventions is being reviewed to include proof of concept. The most advanced initiatives aim to provide public research with the technology needed to reach these objectives. This change has important consequences for the way traditional, public research has been carried out, as well as the way in which it has been financed and evaluated.

In Spain, at both the industrial and academic levels, the current state of affairs lies far from the scenario just described of other countries. This is clearly reflected in the evaluation of how our country scores in terms of innovation, where we find ourselves among the countries that, far from progressing, are actually losing terrain in Europe. Furthermore, while we are among the most important pharmaceutical markets in the world, our contribution to global research and development expenses for pharmaceuticals is just higher than 1%, and the number of innovative drugs of Spanish origin in the global market is extremely limited. Regarding our contribution to scientific production, Spain occupies the 10th position in number of publications, but, again, when translated into patents, our position is far below that which we should be occupying. It's clear that there's an urgent need to adopt reforms in order to correct this situation.

Public research must be provided with the means to participate in a competitive manner in the identification, validation and proof of concept for new therapeutic interventions. Without these instruments, the competitive ability of public research in science would be seriously damaged. Some initiatives should be promoted urgently. For instance, resources such as the collection of biologic samples – above all those of clinical origin – the so-called biobanks, molecular libraries, banks of molecules with biologic activity, biomarkers, molecular imaging, transgenics, cellular and animal models should be made

readily accessible to the academic scientific community. This would represent an important step forward toward increasing the competitive edge of Spain's scientists.

Some recent changes in the funding of research in Spain seem to show changes in this direction, but given our precarious starting point, a more significant effort is important. The Spanish pharmacologists and their col-

leagues from allied disciplines should play a determinate role in this direction. First of all, a cultural change is needed in the researchers, similar to what happened years ago with respect to the publication of results in international journals. A cultural change is also needed regarding the identification, protection, validation and transfer of the research results.

## **Conferences**

# **Diagnostic and Treatment of Complex Diseases**

## Genetic, Epigenetics and Environment in Autistic Spectrum Disorders

*L.A. Pérez Jurado, I. Cuscó, B. Gener, M. Rosales, B. Rodríguez Santiago, F. Gallastegui, M. del Campo*

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Autistic spectrum disorders (ASDs) are behaviorally defined disorders characterized by impairments in social interactions and communication, along with restricted and repetitive behaviors and interest. The prevalence of ASDs was thought to be ~2/10,000 with a male to female ratio of 4:1, but there has been a 10-fold increase in the last 30 years that cannot be exclusively explained by improvements in case ascertainment. There is strong evidence indicating that ASDs have a neurobiological and highly genetic etiology. A comorbid medical condition is present in ~10% of cases, including known genetic disorders. In addition, some ASD cases are caused by chromosomal anomalies (mostly duplications at 15q11-q13 on the maternally inherited chromosome and other rearrangements) or single gene mutations (NLGN3 and -4). However, the specific etiology of the majority of cases remains unknown and genetic studies to date have not uncovered genes of strong effect. The recent finding that our genome is enriched in segmental duplications that behave as hot spots for frequent submicroscopic rearrangements leading to either disease or polymorphic copy-number variants (CNVs) suggests that these variants could be implicated in the cause of a proportion of ASD cases. In addition, environmental influences are considered important in ASDs, as concordance in monozygotic twins is <100%, and the phenotypic expression of the disorder varies widely. An epigenetic component to the disease has been proposed given that environmental factors can influence epigenetic marks and some genomic regions associated with ASDs comprise imprinted domains.

In summary, ASDs are etiologically and biologically heterogeneous with no biological markers available. We propose a mixed genetic/epigenetic model involving genetic mutations and *de novo* genomic rearrangements, along with a combination of several genetic/genomic variants of susceptibility and alterations of epigenetic marks that can be induced by specific environmental factors.

In an attempt to identify the etiology of the disorder, our group is studying a sample of 115 adults and 88 children with a diagnosis of ASD (based on DSM IV criteria, ADIR-positive). Diagnostic studies in all cases include appropriate behavioral and psychological testing, detailed clinical and dysmorphological evaluation, standard karyotype, fragile-X testing, and a rapid detection of genomic rearrangements in the 15q11-q13 (*UBE3A* gene) and 17p11.2 (*RAI1* gene) regions by a multiplex qPCR. Metabolic screening, subtelomeric multiple ligation-probe amplification (MLPA) assays and/or additional tests are also performed when appropriate according to medical records. A questionnaire of pre- and postnatal environmental exposure is also obtained.

For all patients without a confirmed diagnosis, a battery of research studies are being performed, addressed to identify genomic mutations (1-2), epigenetic alterations (4-6), and/or regions and genes of susceptibility through parametric and nonparametric tests in collaboration with groups of the Spanish Network of Psychiatric Genetics ([www.rgpg.net](http://www.rgpg.net)) (7-9):

1. Multiplex qPCR targeting 12 regions on chromosome 7 located between segmental duplications.
2. Array-based Comparative Genomic Hybridization (a-CGH) with a custom-made BAC array (5222 BAC clones covering 23% of the genome, 0.6 Mb average spacing, with high density in putative mutational hot spots – regions flanked by segmental duplications and subtelomeres).
3. Assessment of methylation patterns at the 15q12 and 7q31 imprinted domains by QAMA.
4. Genotypes at functional SNPs in MTHFR and X-inactivation patterns in female patients and patients' mothers
5. Assessment of mono- or biallelic expression with relative allelic expression quantification at 44 genes presumed to be imprinted using 64 SNPs in three multi-

plex Sequenom assays by RT-PCR from lymphocyte RNA.

6. Haplotyping and mutation screening at the mitochondrial DNA.
7. Genotyping of selected CNVs (105 regions containing single copy genes) by MLPA.
8. High throughput semi-automated genotyping of 600 potentially functional SNPs from 200 candidate genes selected on the basis of pharmacology, biochemistry, neuropathology, animal model and linkage data

In the adult group, history and physical exam achieved a diagnosis in 20 patients (17%), and an abnormal karyotype was seen in 13/115 patients (11.3%), all other tests being normal. In the child group, 2 patients were clinically diagnosed of a comorbid condition, 2/88 (2.3%) had an abnormal karyotype, 3/88 (3.4%) had a Fra-X full mutation (1) or permutation (2), and 1 had a deficiency of cerebral creatine. A total of 4/88 (4.5%) cases had an interstitial duplication at 15q11.13. The total diagnostic yield was 25/115 (21.7%) for the adults and 12/88 (13.6%) for the children.

Potentially pathogenic rearrangements have been identified by a-CGH in a significant proportion of cases, which are being characterized in further depth by other methods. QAMA confirmed the maternal 15q11-q13 duplication in the four cases and also identified a patient with an epigenetic mutation at the region resulting in a mosaic pattern (verified by Southern Blot). X-inactivation patterns and MTHFR allele distribution did not differ significantly between patients and controls, and disequilibrium of transmission was not observed. Out of the 51 informative SNPs analyzed of the 38 genes expressed

in lymphocytes by RTPCR, average heterozygosity was 30%. Eighteen genes showed consistent biallelic expression in all cases, while seven were always monoallelically expressed, reflecting their imprinted status. Variable mono or biallelic expression was found in some patients at 13 genes; therefore, they are good candidates for imprinted defects in our autistic sample.

Genotyping of the different genetic variants (mitochondrial haplotypes, CNVs, candidate SNPs) is still ongoing in a larger cohort for association and other family-based studies. Clinical evaluation has allowed us to identify intermediate phenotypes or endophenotypes associated with autism, such as increased head circumference, specific dysmorphology, extreme social difficulties or language impairment. The study of intermediate phenotypes may help to stratify affected cases into more etiologically homogeneous subgroups in order to find common gene variants that lead to autism susceptibility.

In summary, a multistep diagnostic approach to patients with ASDs is able to identify a substantial number of causal diagnoses, with clinical evaluation, karyotyping and analysis of the 15q11-q13 region having a high diagnostic yield. In the remaining patients, the evaluation of genomic imbalances with qPCR and a-CGH has identified several novel putative causes of ASDs. In addition, the finding of epigenetic abnormalities and variability in the pattern of expression of imprinted genes in some cases in an autistic population also suggest that epimutations can be the cause of the disease in a certain number of cases. Along with the pending association studies with genetic and environmental variants, our studies aim at contributing to the knowledge of the complex etiology of ASDs.

## **Lifeguard, Antagonist of FAS in Nervous System**

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The Fas ligand-receptor system plays an essential role in regulating cell death in the developing nervous system, and it has been implicated in neurodegenerative and inflammatory responses in the central nervous system (CNS). Lifeguard (LFG) is a protein highly expressed in the hippocampus and the cerebellum, and it is regulated throughout development in the CNS. Neurons appear to be sensitive to FasL during development and before differentiation. Moreover, LFG shows a particularly interesting regulation by being upregulated during postnatal development and in adults. We show that overexpression of LFG protected neurons from FasL-induced apoptosis and decreased the caspase-

dependent signaling pathway of Fas. Reduction of endogenous LFG expression by small interfering RNA sensitized cerebellar granule neurons (CGNs) to Fas-induced cell death and caspase-8 activation, and also increased sensitivity of cortical neurons. In differentiated CGNs, this resistance appears to be independent of FLIP. Thus, LFG is an endogenous inhibitor of Fas-mediated neuronal death and it mediates the Fas resistance of differentiated CGNs. Finally, we also demonstrate that LFG is detected in lipid rafts microdomains, where it may interact with the Fas receptor and regulate Fas-activated signaling pathways.

## Treatment of Rheumatoid Arthritis Beyond TNF Antagonists

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Most favorable management of rheumatoid arthritis (RA) includes early diagnosis, and treatment with combinations of conventional disease-modifying antirheumatic drugs (DMARDs) and biological DMARDs if these do not induce remission.

Progress in our understanding of the regulatory molecules and pathways that mediate the inflammatory responses in RA has led to the identification of targets for therapy. From the earliest times, it was appreciated that tumor necrosis factor (TNF) played a central role in inflammation by stimulating numerous other mediators of the inflammatory response. Targeting TNF is an effective therapy, with a good efficacy and a good safety profile.

Unfortunately, not all patients respond satisfactorily to TNF inhibition. Blockage of interleukin-1 (IL-1) effects by treating patients with an IL-1 receptor antagonist (anakinra) has not been as effective as expected. A number of other cytokines with proinflammatory activity, including IL-6, IL-12, IL-15, IL-17 and IL-18, have recently been found in the inflamed synovium. Preliminary reports show that inhibition of IL-6 may be a therapeutic alternative for some of those patients failing to respond to TNF antagonists. Studies aiming to block other cytokines are in progress.

B lymphocytes play an important role in the pathophysiology of RA. They stimulate autoaggressive T cells and produce a variety of proinflammatory cytokines that activate monocytes and synoviocytes. The removal of B lymphocytes with rituximab, which recognizes cell-surface CD20 on B lymphocytes in RA, has opened a new therapeutic path in patients responding poorly to conventional disease-modifying drugs or TNF antagonists. Stem cells, plasma cells and memory cells are not affected by

this antibody. This explains, at least in part, why the use of this drug seems to lead to iatrogenic infections less frequently than has been reported for TNF antagonists. Besides CD20 modulation, other cell-surface molecules, such as CD4 and CD154 (CD40L) are also being tested in clinical trials.

Chronic T-cell responses in RA involve many mechanisms. CD28 and cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152) are the primary regulators of T-cell responses. CTLA-4 exerts independent distinct effects during T-cell responses exploited for the treatment of RA. Abatacept (CTLA-4Ig), an antagonist of CD28 costimulation, has been recently approved for the treatment of RA. In clinical trials in RA, its efficacy appears somehow similar to the other biologic drugs.

Identified targets other than T and B cells are adhesion molecules, chemokines, and intra- and extracellular signaling pathways. Chemokines play an important role in the infiltration, localization and retention of infiltrating leukocytes, and the generation of ectopic germinal centers in the inflamed synovium. Recent evidence suggests that identification of inhibitors directly targeting chemokines or their receptors may provide a novel therapeutic strategy in RA. Further drugs being evaluated include stem cell factor receptor or c-kit blockers (imatinib, Glivec), and  $\beta$ -interferon.

In summary, an array of target-oriented drugs, bridging the basic immunology with clinical application, has resulted in marked clinical improvements for many patients with RA. It is expected that these advances will provide significant insights for the treatment of other immune-mediated chronic inflammatory conditions.

## New Therapeutic Targets in Multiple Sclerosis

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Multiple sclerosis (MS) is the most common neurological cause of disability in young people. The cause of MS is unknown, but an immunologic abnormality is suspected. As understanding of the heterogeneous pathophysiology of MS has increased, emphasis has shifted to more selective therapy that targets components of the inflammatory cascade and the promotion of remyelination and neuroprotection. These agents target the blood-brain barrier, systemic immune dysfunction, local inflammation and neurodegeneration. Many new drugs are being developed and tested that address these issues with the aim of finding a more effective and convenient therapy. These include humanized monoclonal antibodies such as natalizumab (anti- $\alpha$ 4 integrin) (1) and daclizumab (IL-2 antagonist) (2), oral immunomodulators such as fingolimod (FTY-720) (3), and agents involved in remyelination (anti-LINGO-1) (4) and neuroregeneration acting in the Nogo receptor (5). Some of the treatments discussed are still in early stages of development but provide exciting potential treatment options; others have proved encouraging in larger extended-phase studies.

During the last 10 years, a series of new therapeutic approaches have been developed. The monoclonal antibody natalizumab (1) has recently been approved in the United States and the European Union, with some restrictions due to its safety profile.

A clinical phase III study is being planned for daclizumab, which has shown to limit T-cell expansion by blocking interleukin (IL) 2 signaling by means of its high-affinity receptor that is expressed on activated T cells (*i.e.*, blocking IL-2R $\alpha$ -chain, CD25). Daclizumab inhibits solid-organ graft rejection and helps to restore tolerance in immune-mediated uveitis. Based on analo-

gies of pathogenesis between these conditions and aberrant T-cell activity in MS, the effect of add-on therapy of daclizumab in MS patients with incomplete clinical and MRI response to interferon- $\beta$  therapy was tested with positive results (2).

Fingolimod is an orally active immunosuppressant under development for use in transplantation and autoimmune diseases. It reduces the number of lymphocytes in the blood by redirecting them to the lymph nodes. The results in experimental allergic encephalomyelitis, the animal model for MS, suggested that the protective anti-inflammatory effect of treatment with fingolimod was, to a large extent, due to the inhibition of encephalitogenic T-cell responses and/or their migration into the central nervous system. A phase II study in MS patients has shown promising results, and the drug will continue its development in phase III studies (3).

The experimental work on neuroprotection in animal models of inflammatory axonal degeneration and consideration of outcome measurement in MS have developed sufficiently to enable trials of neuroprotection to be planned, powered and implemented. Trials assessing neuroprotection with tetrahydrocannabinol and lamotrigine are imminent; both will involve subjects with progressive forms of MS (6).

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# **Innovation Therapeutics**

## Strontium Ranelate: Is it Possible to Uncouple the Bone Turnover?

R. Moruno

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Various bone resorption inhibitors have been shown to decrease the risk of osteoporotic fractures. However, there is still a need for agent promoting bone formation by inducing a positive uncoupling between bone formation and bone resorption. Strontium ranelate is the first treatment to stimulate bone formation while decreasing bone resorption. *In vitro* studies showed that strontium ranelate stimulates the replication for preosteoblastic cells and collagen synthesis in rat calvaria culture and isolated cell. Strontium ranelate was also found to inhibit the bone-resorbing activity of isolated rat and mouse osteoclasts, as well as osteoclast differentiation in chicken bone marrow cultures. In ovariectomized rats, strontium ranelate decreased bone resorption while bone formation was maintained, preventing trabecular bone loss induced by estrogen deficiency. In normal rat, strontium ranelate was demonstrated to stimulate alkaline phosphatase activity, a marker of bone formation. The significant marked increment of trabecular bone volume, trabecular number and increment of bone diameter are further elements in favour of an *in vivo* stimulation of bone formation.

These effects have also been shown in the biochemical markers weighed up within osteoporotic women in SOTI study, where an increase in alkaline phosphatase and a decrease in serum CTX were observed. In addition, a recent study about human bone biopsies of transiliac bones, has highlighted that strontium ranelate significantly increases formation parameters and decreases the resorption ones. This data confirms what it had already been shown in clinic, *in vivo* or *in vitro*.

In intact female rats, two-year exposure to strontium ranelate mixed in the diet induces a dose-dependent increase in bone strength and bone mass at the level of the vertebral body, which contains a large proportion of trabecular bone, and at the level of the midshaft femur, which mainly contains cortical bone.

This data indicates that strontium ranelate stimulates bone formation, inhibits bone resorption, and increases bone mass, resulting in bone strength improvement.

The efficacy of strontium ranelate, 2 g daily, in postmenopausal women with osteoporosis has been investigated in 2 large randomised, double-blind, placebo-controlled trial, namely SOTI (Spinal Osteoporosis Therapeutic Intervention) and TROPOS (Treatment of Peripheral Osteoporosis).

These studies were preceded by a run-in period, the main aim of which was to start the normalization of calcium and vitamin D status. The primary end point of SOTI was reduction in vertebral fractures and, in TROPOS, reduction in nonvertebral fractures. The number of women recruited was 1649 and 5091m respectively, with a mean age of 69 years in SOTI and 77 years in TROPOS.

In SOTI, strontium ranelate therapy was associated with a significant reduction in vertebral fractures, with a 41% reduction in relative risk (RR) over 3 years, the fractures incidence being 32.8% in the placebo group and 20.9% in the treatment group. This beneficial effect was seen after only 1 year of treatment (RR 0.51; 95% confidence interval [CI], 0.36-0.74). There was also a significant reduction in clinical vertebral fractures (RR 0.62; 95% CI, 0.29-0.80) and in the height loss ( $P = 0.003$ ) at 3 years. Pooled subgroup analyses of patients from SOTI and TROPOS demonstrated significant reductions in vertebral fractures risk in women with or without vertebral fractures at baseline, women with osteopenia, and women aged over 80 years.

A significant reduction in nonvertebral fractures was demonstrated in TROPOS, with a relative risk of 0.84 (95% CI, 0.702-0.995) in treated women. In a post hoc analysis of osteoporotic women aged over 74 years, a significant 36% reduction was seen in hip fracture risk (RR 0.64; 95% CI, 0.412-0.997) over 3 years.

The long term efficacy of strontium ranelate has been also demonstrated over 4 and 5 years.

In the SOTI study, the long term efficacy data confirmed a significant reduction in the risk of new vertebral fractures by 33% in the strontium ranelate group ( $n = 719$ ) as compared to the placebo group ( $n = 723$ ) in

the intention to treat population over the four years treatment period (RR=0.67; 95% CI [0.55;0.81], Cox model:  $p < 0.001$ ).

The 5 years result of TROPOS established the long term efficacy of strontium ranelate, with a 24% reduction in vertebral fractures (RR = 0.76; 95% CI [0.65;0.87] Cox model:  $p < 0.001$ ) and a 15% reduction in nonvertebral fracture (RR = 0.85; 95% CI [0.77;0.99] Cox model:

$p = 0.03$ ) in the intent to-treat population (n = 2479 in strontium ranelate) group and 2453 in placebo group).

These results demonstrate, uniquely for anti-osteoporotic treatment, that strontium ranelate provide sustained efficacy over five years against both and nonvertebral fractures. Its therapeutic spectrum makes it an alternative first-line option to bisphosphonates in the prevention of osteoporotic fractures in postmenopausal women.

## Insulin Detemir: A New Basal Insulin Analog for the Treatment of Diabetes

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Insulin detemir (IDe) is a soluble long-acting insulin analog that provides more predictable and consistent control of blood glucose levels. IDe is made by altering the chemical structure of insulin to allow more consistent release during the day, thereby mimicking natural basal insulin release. IDe is produced by a process that includes expression of recombinant DNA in *Saccharomyces cerevisiae* followed by chemical modification. IDe differs from human insulin in that the amino acid threonine in position B30 has been omitted, and a C14 fatty acid chain (myristic acid) has been attached to the amino acid B29. The molecule has a fatty acid attached, enabling it to bind to albumin within the subcutaneous tissue and bloodstream and release IDe at a slow, consistent rate. This unique method of prolonging action is known as protraction. The combination of protracted absorption from the injection site and delayed action due to albumin binding provides a prolonged and predictable action profile with low within-subject day-to-day variation and a reduced risk of hypoglycemia. The mean duration of action of IDe ranged from 5.7 hours at the lowest dose to 23.2 hours at the highest dose. After subcutaneous injection of IDe in healthy subjects and in patients with diabetes, IDe serum concentrations indicat-

ed a slower, more prolonged absorption over 24 hours in comparison to NPH human insulin. The absolute bioavailability of IDe is approximately 60%, and more than 98% in the bloodstream is bound to albumin. In clinical studies, the effect of IDe as a basal insulin is better in comparison with NPH in terms of achieving glycemic control. There is a lower within-subject variability of insulin effect with IDe than with NPH or insulin glargine, thus making the effect more predictable. In comparison with NPH insulin, IDe was associated with less weight gain and fewer hypoglycemic events, especially nocturnal ones. Therefore, IDe could be particularly suited to people with diabetes who have problems with erratic blood glucose control, who experience hypoglycemia (especially at night), or who find it difficult to manage their weight once blood glucose control improves.

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## **Efalizumab: The New Therapeutic Option for Chronic Plaque Psoriasis Using Recombinant DNA Technology**

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Psoriasis is a common, persistent, inflammatory autoimmune disorder with cutaneous manifestations that can have a major effect on patient quality of life. For several decades conventional psoriasis treatments have failed to meet clinical needs for a safe and effective therapy. Recent research has demonstrated that psoriasis is not the result of a keratinocyte disorder, as previously believed, but in fact the implication of an immunological T-lymphocyte phenomenon in the pathogenesis of psoriasis has led to research for new treatment options over the past few years. Innovations in biotechnology have made possible the development of several new systemic therapies for psoriasis – the “biologicals,” a new group of compounds including monoclonal antibodies, fusion proteins and recombinant proteins – that target the immune system. These new biologic drugs-proteins are synthesized using recombinant DNA technology to naturally mimic molecules that selectively target the immune system. Certainly these novel biotechnological advances have changed the paradigm for treating this disease. Efalizumab (Raptiva) is a new therapy that offers patients with psoriasis the potential for safe and effective long-term management of plaque psoriasis. It is a recombinant humanized monoclonal IgG1 antibody against CD11a, the  $\alpha$ -subunit of leukocyte function-associated

antigen 1 (LFA-1). LFA-1 and intercellular adhesion molecule 1 (ICAM-1) are costimulatory molecules expressed on T cells and antigen-presenting cells, respectively, that facilitate multiple T-cell mediated events. By interfering with LFA-1, Efalizumab inhibits multiple steps in the immune cascade that result in the production and maintenance of psoriatic plaques, including initial T-cell activation in the lymph nodes, trafficking of T cells from the circulation into dermal and epidermal tissue, and T-cell reactivation in those sites. Results from three phase I studies have demonstrated that there was a decrease in epidermal and dermal T-cell infiltrates in biopsies with Efalizumab, suggesting that the optimal subcutaneous (s.c.) dose of Efalizumab is 1 mg/kg/week. Dosages greater than 1 mg/kg/week s.c. did not provide additional benefits; moreover, higher doses did not alter the safety profile. Clinical studies have demonstrated that Efalizumab is efficient and safe in the treatment of chronic plaque psoriasis. Approximately 30% of patients achieve an improvement in PASI of  $\pm 75\%$  within 12 weeks, with further clinical benefit noted with continued therapy up to three years.

The field of dermatology has now entered a new era, joining other disciplines of medicine that have been using biologic agents for decades.

## Innovation in the Treatment of Secondary Hyperparathyroidism: Cinacalcet, a New Therapeutic Class

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The standard treatment for secondary hyperparathyroidism (vitamin D and phosphorus binders) fails to achieve adequate control; on the contrary, it maintains elevated concentrations of parathyroid hormone (PTH) and phosphorus, which are, in turn, associated with a higher risk of mortality and hospitalization in dialyzed patients with stage 5 chronic kidney disease. In fact, in recent years, the mortality rates for these dialysis patients have not improved.

Mimpara® (cinacalcet) is the first drug of a new therapeutic class known as antiparathyroid agents. This drug is a calcimimetic agent that binds to the calcium-sensing receptor (CaR) of the principal cells of the parathyroid glands at a position different from that of calcium, and enhances its sensitivity to extracellular calcium. The activation of CaR results in a decrease of the serum concentrations of PTH as well as a decrease in serum calcium and phosphorus.

The therapeutic indications of Mimpara® are secondary hyperparathyroidism in patients with chronic kidney disease in dialysis, and hypercalcemia in patients with parathyroid carcinoma. According to the patient's

situation, Mimpara® can be used in monotherapy or as part of a therapeutic regimen that includes phosphorus binders and/or vitamin D analogs.

Mimpara® is the only available treatment that successfully reduces PTH levels while simultaneously decreasing serum levels of calcium, phosphorus and calcium-phosphorus product (Ca×P). Consequently, it improves the clinical outcomes of patients with SHPT. Mimpara® significantly reduces the risk of parathyroidectomy, fractures and cardiovascular hospitalizations. Mimpara® improves the prognosis of these patients; moreover, its efficacy is independent of disease severity, baseline levels of Ca×P, time of dialysis and use of other therapeutic options. In addition, Mimpara® achieves control of SHPT with lower therapeutic doses of vitamin D; therefore, negative secondary effects of vitamin D, such as hypercalcemia and hyperphosphatemia, can be avoided. The field of dermatology has now entered a new era, joining other disciplines of medicine that have been using biologic agents for decades.

## Anagrelide: A Novel Drug in the Treatment of Thrombocytosis

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Platelet-lowering therapy in myeloproliferative disorders includes cytostatic drugs, mainly hydroxyurea, interferon alpha and anagrelide. Anagrelide (Agrylin, Xagrid), an oral imidazoquinazoline agent, is the latest addition to the therapeutic arsenal. Anagrelide is well established as an effective platelet-lowering agent in most patients with essential thrombocythemia, including both treatment-naïve patients and those refractory to other cytoreductive therapy. The initial adult dose is 0.5 mg p.o., taken three or four times daily. This dose is then adjusted according to the platelet count response and symptomatology. The usual maintenance dose is in the range of 1-4 mg/day p.o.

The platelet-lowering efficacy is 70-80% in essential thrombocythemia, and the response is rapid; most patients reach the treatment goal within a few weeks. Toxicity of anagrelide is mainly related to the drug's direct vasodilatory and inotropic effects. Side effects include headache, palpitations/tachycardia, fluid retention and diarrhea. A reduction in hemoglobin concentration of more than 3 g/dl occurs in 24% of cases following long-term treatment.

Results of the only randomized trial to date (the Primary Thrombocythaemia 1 [PT1] study) indicated that the composite primary endpoint (arterial or venous thrombosis, serious hemorrhage or death from vascular causes) occurred more often in recipients of anagrelide plus aspirin than in those receiving hydroxycarbamide (hydroxyurea) plus aspirin. This trial also indicated that the incidence of the secondary endpoints transient

ischemic attack and gastrointestinal bleeding favored hydroxycarbamide plus aspirin, while the incidence of venous thrombosis favored anagrelide plus aspirin. There were no differences between the groups in the incidence of secondary endpoints myocardial infarction, stroke, unstable angina, pulmonary embolism, hepatic-vein thrombosis, other serious hemorrhage or related deaths. The design of the PT1 study has been queried with respect to the heterogeneous nature of the study population (possible inclusion of patients with early myelofibrotic disease) and the concomitant use of aspirin (interaction with anagrelide causing increased bleeding events). More insights are expected from the recently completed ANAHYDRET trial that compared monotherapy with hydroxyurea and anagrelide. This drug has recently been registered in Europe as a second-line therapy in essential thrombocythemia but is often used as first-line therapy in the United States, especially in younger patients, due to the concern about increased leukemia risk with cytostatic treatment.

Several groups of investigators have studied the mechanism of anagrelide-induced platelet underproduction in the various myeloproliferative disorders. Their observations suggest drug interference with megakaryocyte proliferation and maturation, resulting in platelet underproduction. The platelet function inhibitory activity of anagrelide is seen only at doses higher than those used for controlling thrombocytosis and should not be a concern in patients with essential thrombocythemia.

