Resolution Pharmacology: therapeutic innovation in inflammation.

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Current medicines for the clinical management of inflammatory diseases act by inhibiting specific enzymes or antagonising specific receptors or blocking their ligands. In the past decade, a new paradigm in our understanding of the inflammatory process has emerged with the appreciation of genetic, molecular and cellular mechanisms that are engaged to actively resolve inflammation. The 'resolution of acute inflammation' is enabled by counter-regulatory checkpoints to terminate the inflammatory reaction, promoting healing and repair. It may be possible to harness this knowledge for innovative approaches to the treatment of inflammatory pathologies. Here we discuss current translational attempts to develop agonists at pro-resolving targets as a strategy to rectify chronic inflammatory status. We reason this new approach will lead to the identification of better drugs that will establish a new branch of pharmacology, 'resolution pharmacology'.

What goes up must come down

The observation that novel bioactive mediators could be synthesised within exudates at the peak of inflammation set in motion a major conceptual shift in our understanding of the biological control of the host response to injurious agents. These mediators, termed lipoxins, are downstream products of arachidonate metabolism, generated through a then novel trans-cellular mechanism, requiring a two-step biosynthetic pathway with enzymes brought into close vicinity by two distinct cell types (1, 2). Lipoxins were the first factors demonstrated to inhibit leukocyte trafficking and promote apoptosis and phagocytosis of apoptotic cells (or efferocytosis) (3) and were highlighted in a 1997 *TiPS* review on 'endogenous inhibitors of leukocyte trafficking' (4). Over the last 15 years this field of research has burgeoned with, on one side, the identification of several mediators synthesised in a strict temporal and spatial fashion to actively prevent the over-shooting of acute inflammatory mechanisms; on the other side, specific processes have been detailed and found to impact on this counter-regulation on the host response. Thus, appreciation of the complexity of the actions evoked by endogenous mediators of protection, and the implications of this biology on the well-being of the host, has led to the definition of the 'resolution of inflammation', with a consensus review in 2007 (5): therein we defined the principal features of resolution, its processes and mediators. **Figure 1** schematizes the process of acute inflammation, depicting an onset phase followed by a resolution phase. Indeed, likely too simplistically, curves indicating an upward trajectory followed by a downward one (going up and coming down) are typically used to represent the time-profile of the acute inflammatory response. A balanced reaction is the ideal response for the host (to be further discussed below; see also **Figure 1**).

The Resolution of Acute Inflammation

The past few years have witnessed an increment of interest in this field as testified by the number of cutting edge reviews that have detailed the biology of specific pro-resolving mediators and/or the processes of resolution (e.g. see (4, 6-10)). Herein, we summarise salient aspects of resolution especially if relevant to further discussion; the reader is re-directed to these reviews for deeper analyses on mechanisms, as our scope is to reason on the impact that this area of science is having on informing the development of novel anti-inflammatory agents.

Mediators of Resolution

Similarly to the onset phase of inflammation, pro-resolving mediators encompass bioactive lipids (e.g. lipoxins, resolvins), proteins and peptides (e.g. adrenocorticotropic hormone, annexin A1, chemerin peptides, galectin-1), autacoids (e.g. adenosine) and gases (e.g. H_2S and CO). Mediators of resolution share fundamental properties to terminate the inflammatory reaction and organise the 'cleaning phase' within the affected tissue, as required for the regain of homeostasis and return to normal physiological function. We and others have defined the bioactions that qualify a pro-resolving agonist; these include i) inhibition of granulocyte trafficking; ii) non-phlogistic migration of monocytes; iii) promotion of granulocyte apoptosis; iv) augmentation of phagocytosis (bacteria containment) and efferocytosis; v) control of resident cell phenotype (e.g. M1 to M2 macrophage switch) (6, 8, 11) and vi) promotion of tissue regeneration and repair (12-14). **Figure 2** highlights some of the biological properties of pro-resolving mediators that can be harnessed for Resolution Pharmacology, as compared to those of therapeutics targeting effectors of inflammation. One of the main differences is that the aim of Resolution Pharmacology is to balance the inflammatory response, allowing pro-inflammatory mechanisms to exert their life-saving functions. For example, in experimental myocardial infarct, an excess of matrix metalloproteinase-12 (MMP-12) can be damaging but its complete inhibition is equally detrimental as it prolongs neutrophil survival leading to extensive tissue damage (15). An MMP-12 inhibitor in this model would then be resolution-toxic.

The biology of pro-resolving mediators is quite varied both in terms of cell sources, biosynthetic pathways (e.g. the two-step biosynthesis of lipoxin A_4) and the engagement of specific targets. In **Box 1** we report the "identity card" of exemplar pro-resolving mediators to illustrate the breadth of biological processes that are required for their expression and function. As an example, the glucocorticoid-regulated protein annexin A1 (AnxA1) is expressed within the cytosol of resting cells (myeloid cells have high AnxA1 levels, but also

epithelial and endothelial cells express this protein) probably staying in *a silent mode* (16). Upon cell activation, AnxA1 is externalised through non-conventional pathways that may involve vesicle budding from the plasma membrane. Within the extracellular fluid, AnxA1 acquires an active conformation to agonise a specific G-protein coupled receptor (GPCR) termed FPR2/ALX through its N-terminal region (~50 amino acids). Therefore, cell stimulation brings about AnxA1 control of inflammation: in AnxA1 null mice or animals nullified for its receptor an overshooting of the inflammatory response is observed, as evident in models of arthritis (17, 18) and colitis (13, 19). Intriguingly, the bioactive lipid mediator lipoxin A_4 (LXA_4) which is synthesised on demand when two cells are in close contact, bringing together the 5-lipoxygenase and 12- or 15-lipoxygenase (e.g. neutrophil and platelet, or macrophage and epithelium) also activates FPR2/ALX, the same receptor for AnxA1. However, there is distinction in the production of these endogenous agonists; hence, there are mechanisms of modulation and balance, which represent the essence of the endogenous control on the inflammatory response. To further surprise, when myeloid cells use docosahexaenoic acid (DHA) instead of arachidonate, synthesis of resolvin D1 (RvD1) can be obtained, another agonist for FPR2/ALX. **Box 1** also reports the "identity card" for alpha-melanocyte stimulating hormone (αMSH), originally identified as a skin-darkening hormone but in reality endowed with multiple biological properties including potent modulation of immune cell activity and immune processes (20, 21).

Adenosine, a purine nucleoside composed of a molecule of adenine attached to a ribose sugar molecule, is a pro-resolving autacoid. Extracellular adenosine signals through adenosine receptors and a number of studies suggest a crucial role for this mediator particularly in lung physio-pathology (22, 23). Adenosine levels increase in areas of inflammation and hypoxia, the major source being the metabolism of intracellular ATP, ADP and AMP by the action of 5'-nucleotidases. Once released adenosine activates specific receptors to re-balance immune cell reactivity: these cells include neutrophils, macrophages and T cells, thus enacting the processes of resolution discussed above (23-25).

While adenosine is produced locally, systemic hormones have also been identified as genuine pro-resolving mediators. Examples are αMSH and adrenocorticotrophin (ACTH). Presence of these hormones into exudates could result from local synthesis that may take place within a multicellular tissue altered by presence of inflammatory cells; more canonically, plasma extravasation would carry the mediator to the exudate hence to the site of inflammation. In any case, αMSH levels are elevated in synovial fluids from patients from rheumatoid arthritis compared with osteoarthritis (26). In addition, the concentration of αMSH in synovial fluid is greater than in plasma, suggesting either local production or the existence

of processes of enrichment. Interestingly, plasma αMSH is also elevated in ischemic stroke and appears to predict long-term outcome (27).

Pro-resolving receptors

The functions of pro-resolving mediators are transduced by a variety of receptor types, similarly to the onset phase of inflammation. Thus, membrane receptors (including GPCRs immunoglobulin-like receptors, and tyrosine kinase receptors) and cytosolic receptors can convey pro-resolving effects. Cytosolic receptors encompass the receptors for steroid hormones, which include sex steroids and adrenal steroids, as well as members of the metabolic nuclear receptor family such as PPARs, FXR, LXR (28, 29). Below we will focus on GPCRs (FPR2/ALX, melanocortins and adenosine receptors in particular) in view of their versatility and druggability, and the ability of these receptors to interact with ligands of distinct nature. We conclude by highlighting the potential of this new approach.

Inflammation has emerged as a critical process in the pathogenesis of numerous chronic diseases, from those classically described as inflammatory pathologies (e.g. rheumatoid arthritis (RA), gout, asthma) to those previously unappreciated for their inflammatory aetiology or component (e.g. osteoarthritis, atherosclerosis, Alzheimer's disease or cancer) (30). In addition, comorbidities associated with obesity, such as metabolic syndrome and type 2 diabetes, are the consequence of a persistent "low grade" inflammatory state triggered by nutrient metabolic surplus (referred to as metabolic-triggered inflammation or "metainflammation") (31). Appreciation of endogenous resolution has two immediate consequences:

First, the chronicity of inflammatory diseases can be associated to inadequate engagement of resolving pathways (7). For example, inadequate phagocytosis of apoptotic cells, a key pro-resolving mechanism, is defective in atherosclerotic plaques, leading to the formation of the necrotic core and eventually plaque disruption (32, 33).

Second, therapeutic innovation can derive from harnessing the tissue protective properties of resolution (34). We propose that identification of specific pro-resolving receptor signatures should be applied to drug discovery programmes to yield a pro-resolving lead compound.

The definition of resolution pharmacology

How could we harness resolution mediators, targets and processes to establish resolution pharmacology? Broadly, there are three distinct approaches amenable for exploitation: i) to

mimic endogenous pro-resolving mediators; ii) to develop agonists at pro-resolving receptors and iii) to potentiate endogenous pro-resolving pathways.

Mimetics of effectors of resolution

ACTH has long been known to be effective in controlling clinical signs of arthritides, including RA and gout. This work has been validated in controlled clinical studies including hospitalised patients suffering from severe gout (21, 35). ACTH is being trialled for efficacy in multiple sclerosis and systemic lupus erythematosus (**Table 1**). Modifications of αMSH, a 13 amino acid peptide derived from ACTH, have inspired a series of peptides trialled in distinct settings. Recently, a synthetic analog of αMSH (afamelanotide or Scenesse®) has been approved by the European Medicine Agency (EMA) for erythropoietic protoporphyria. This represents a success for melanocortin biology (36). The skin darkening associated with melanocortin peptides is often perceived as an unwanted side effect by these peptides, but it is clearly beneficial in a condition characterized by light intolerance. More examples of drug development approaches based on melanocortin peptides are reported in Table 1.

LXA_4 analogues have been developed for some time, aiming at more stable molecules. A benzo-LXA_4 derivative recapitulates all the pro-resolving properties of lipoxin in a translational model of periodontitis in the pig (37) demonstrating tissue-regenerative properties. The fact that the compound was encapsulated in vesicles to generate nanomedicines opens a new avenue for exploiting the bio-actions of pro-resolving mediators: nanomedicines would not only correct unfavourable pharmacokinetics but also improve targeted delivery. The nanomedicine approach has been highly successful in complex experimental settings of disease using synthetic targeted nanoparticles fortified with a bioactive peptide derived from AnxA1 (38, 39). Table 1 lists examples where the pro-resolving properties of bioactive lipids like LXA_4 or RvE1 are being tested in humans. In some cases, quantification of pro-resolving mediators is being validated as a marker of distinct treatments aiming at boosting endogenous tissue-protection.

Adenosine is currently used for the management of arrhythmias, although its clinical utility is limited due to its short half-life (< 10 seconds). Stable analogues targeting the adenosine receptor A_{2A} have been developed and tested in clinical trials (40). For example, compounds UK-432097 and sonedenoson (MRE-0094) both reached Phase II studies for the treatment of asthma and diabetic foot ulcers, respectively. Adenosine analogues in the form of pro-drugs are also under development as level of the enzyme responsible of its activation (ecto-5'-nucleotidase) are higher at sites of inflammation, reducing potential side effects such as hypotension. Activation of the receptor A_{2B} also plays a role in inflammation (41) and it has

been suggested to mediate, at least in part, the tissue protective actions of ischemic pre-conditioning procedure. More examples are presented in Table 1.

Agonists at pro-resolving receptors

There are no doubts that the most palatable approach to the pharmaceutical industry is the one based on the identification and development of orally-active small molecule therapeutics. Unmanned high-throughput screenings have allowed the search for GPCR agonists even at receptors naturally activated by peptides (the dogma being that a peptide binding site is too big to be 'modelled' by a small chemical entity). Whilst the complexity of pro-resolving GPCRs can represent an initial barrier to selection as innovative molecular targets, this complex biology provides an opportunity for drug discovery: distinct ways are being explored to achieve GPCR agonism and these include identification and development of orthosteric agonists, allosteric agonists or modulators, biased ligands as well as bi-topic agonists (42, 43). **Box 2** schematises some of the strategies that we see viable for resolution anti-inflammatories targeting pro-resolving GPCRs.

As an example, we focus here on FPR2/ALX (formyl peptide receptor type 2; lipoxin A_4 receptor) as this receptor is emerging as a master receptor of resolution. Preclinical evidence generated with pharmacological studies as well as data obtained with transgenic mice indicate fundamental pro-resolving and protective properties downstream FPR2/ALX activation. These non-redundant functions of FPR2/ALX derive from its ability to bind to, and transduce signals from, a relatively large series of agonists (44). In 2002 we identified this receptor as the first one to ligate both bioactive lipids and peptides/proteins, suggesting even at that time its potential for controlling multiple facets of the inflammatory reaction (45). Modelling studies on the receptor binding sites, together with analyses using series of chemical structures, have led to a topography with three main sites of interaction/interlocking of small molecule agonists (46), confirmed in side-by-side analyses when comparing small molecule with peptide agonists (47). The complexity of the biology of FPR2/ALX is not limited to the wealth of ligands that can bind (though it remains fundamental to establish which are genuine agonists during on-going inflammatory responses) but also to the recent appreciation that FPR2/ALX dimers can be activated in a biased fashion, that is only by pro-resolving agonists (48).

We have recently reviewed the pharmaceutical efforts in developing new chemical entities at FPR2/ALX (49). Multiple approaches have been applied over the years, encompassing peptide analogues or lipoxin analogues, as well as small molecule development, indicating that much progress since the first putative selective FPR2/ALX agonists reported by Amgen

(50, 51) has taken place. In **Table 2** we present the result of a patent search on small molecule FPR2/ALX agonists as conducted in May 2015, demonstrating the fertility of activities within this area of biology and the genuine attempts to harness the pro-resolving properties of this master-receptor of resolution,

Small molecule agonists at melanocortin receptors have also been developed. Receptor subtypes MC_1, MC_3 and MC_5 present particular interest as anti-inflammatory targets. The molecule BMS-470539 is a potent and selective MC_1 agonist (52). Its chemical structure mimics the core amino acid peptide sequence His-Phe-Arg-Trp, which is common to all melanocortin peptides and crucial for activity. Pre-clinical studies have shown protective actions in animal models of experimental nephropathies and vascular inflammation (53, 54). Interesting work performed on melanocortins pointed out the importance of considering single nucleotide polymorphism (SNP) variants in the drug development process, in particular for receptors encoded by highly polymorphic genes such as MC_1, as these variants can differentially alter the signalling cascade evoked by a ligand (55). BMS-470539 acts as a classical orthosteric agonist. However, we recently described a new molecule that benefits from the complexity of GPCR biology (see Box 2). AP1189 is a small molecule that acts as a biased agonist, as it does activate ERK1/2 and Ca^{2+} pathways but not the canonical cAMP. The relevance of this unusual activity is that the side effects associated with skin darkening (MC_1-cAMP dependent) are avoided (56).

A new pro-resolving mechanism emerged after the recognition that immune functions can be modulated by the interaction of endocannabinoids with specific receptors expressed in immune cells, predominantly CB_2, by inhibiting leukocyte migration and cytokine release, among other actions (57). Phase II clinical trials have been conducted with Resunab (ajulemic acid, CT-3, IP-715) for the treatment of neuropathic pain, showing effectiveness and no major side effects (58). This CB_2 agonist is also under development or the treatment of cystic fibrosis and diffuse scleroderma.

An important concept we wish to highlight here is that identification of the ‘correct’ receptor target may not be sufficient to inform successful drug discovery programmes for resolution pharmacology. The versatility of GPCRs, for instance, favours complexity but also provides an opportunity. In Box 2 we schematize possible approaches to agonist development. Furthermore, we propose that selection of the ‘appropriate pro-resolving signalling’ is of paramount importance. For instance, many pro-resolving GPCRs can signal through elevating intracellular calcium, yet this readout may be of little prediction for novel pro-resolving-based therapeutics. In a recent work with FPR2/ALX and melanocortin receptors,

we have provided experimental substance to this concept (48, 56). For many GPCRs homo- and hetero-dimerization can incite specific signalling responses with ensuing biological properties. Therefore, Identification of the *pro-resolving signature evoked by specific effectors of resolution* ought to guide the development on small molecule agonists for these to result endowed with the wanted pro-resolving biological actions.

Boosting endogenous pro-resolving pathways

The most natural way to boost patient specific endogenous tissue-protection derives from the dietary delivery of omega-3 fatty acids. The beneficial effects of fish oil supplementation have often been reported sporadically, but a recent meta-analysis on omega-3 has concluded on the clinical beneficial efficacy in joint diseases (59). Clinical experimentation on a convenient drink delivering omega-3, versus classical EPA/DHA supplementation, has been conducted in atherosclerosis, using a patient age range of 30 to 75 year old, and an 8-week dose regimen (NCT00886704). The results demonstrated that supplementation augmented circulating omega-3 levels without any evident sign of toxicity (60). **Table 1** highlights a selection of omega-3 based dietary delivery with clear clinical outcomes. It remains to be demonstrated that following these supplementations, conversion to bioactive levels of resolvins and protectins occurred within the biological fluids of these volunteers or patients.

A serendipitous way to augment endogenous players of resolution was discovered following the observation that widely used drugs like aspirin and statins can alter the catalytic properties of the cyclooxygenase by preventing prostanoid generation yet leading to the synthesis of epimeric versions of LXA_4 or, if DHA is the substrate fatty acid, RvD1. In an investigational proof-of-concept study low dose, but not full dose, aspirin administration to volunteers augmented epi-LXA_4 levels (61). More recently similar effects of statins have been recognized (62). For example, lorvastatin can increase the formation of 15-epi-LXA_4 by human neutrophils co-incubated with airway epithelial cells (63). On a similar vein, we have observed that high dose glucocorticoid treatment to patients suffering from giant cell arteritis (a vascular inflammatory condition with great morbidity, as the inflammation within the temporal artery can lead to blindness) incremented AnxA1 expression on circulating neutrophils, perhaps representing a biomarker if not a genuine effector of the clinical efficacy of the steroids (64).

A different strategy that can yield promising results attempts to exploit the ubiquitous gasotransmitters. In the case of hydrogen sulfide, its endogenous levels can be boosted by using N-acetyl-Cysteine, which has already been trialled in humans (Table 1), or with novel

chemical entities, as recently reviewed (65). Similar approaches have been taken for nitric oxide releasing molecules or carbon monoxide releasing molecules (e.g. see (66)). Of interest, inhaled carbon monoxide elevates LXA_4 and resolvin series E levels in the plasma of infected baboons, a response associated with shortening of the interval for pneumonia resolution (67). Finally, in line with the interest in fish oils and omega-3 fatty acid supplementation, it is noteworthy how the nitrite-rich beetroot juice can augment endogenous levels of nitric oxide, through biochemical pathways that involve mouth bacteria, and reduce systemic blood pressure. Following a series of pre-clinical experimentations, this biochemical pathway has been recently demonstrated in a Phase II randomized control trial (68) (Table 1).

Promotion of apoptosis has long been advocated as a successful pharmacological strategy to temper excessive recruitment of leukocytes (neutrophils or eosinophils, depending on the pathology) into vital tissues, such as the lung of chronic obstructive pulmonary disease or asthmatic patients (69). There is a wealth of preclinical work that indicates how the pro-resolution approach can be exploited to re-direct over-exuberant inflammatory responses (70) and this may be linked mechanistically, for example, to inhibition of cyclin-dependent kinases (71). Similarly, still within the preclinical arena are approaches based on small interference RNAs: these could be exploited for positive modulation of pro-resolving mediators or targets or for negative modulation of inhibitors of their expression, the ultimate outcome being boosting of specific pro-resolving pathways. For example, low levels of microRNA-181b upregulate FPR2/ALX expression in human macrophages (72). In mouse macrophages, miR-466l overexpression increases levels of RvD1 and RvD5 with a positing effect on the kinetics of resolution (73).

Therapeutic areas where pro-resolving based drugs could impact

The development of resolution pharmacology we discuss herein will afford creation of another option for clinicians to keep under check chronic inflammatory pathologies. We do not advocate that pro-resolving drugs will replace current therapeutic options but certainly we envisage that they could act as complement. In settings of arthritis, for instance, following a therapy with a biologic and methotrexate to keep under control the florid phase of the disease (74), a resolution-based therapy could be applied for maintenance *and* promotion of tissue (synovia, cartilage) repair. Indeed, experimental work demonstrates that pro-resolving mediators are able to ‘revert’ or prevent cartilage damage (75, 76). Interesting tissue-reparative properties have been reported for AnxA1 (38), in analogy to recent studies on tissue regeneration with maresins (77). Novel 14-sulfido-conjugated maresins, characterised by presence of glutathione or cysteine, are also able to promote tissue regeneration (78). It

cannot be excluded an alternative therapeutic use, with administration of a pro-resolving drug early during the course of disease to *push return to homeostasis* thus enhancing the chances of efficacy of classical anti-inflammatory drugs.

Resolution pharmacology may represent a viable therapeutic option for complex diseases that are currently poorly managed, with sepsis and Alzheimer's disease being the exemplars. Classical anti-inflammatory therapies have failed, if not been *tout-court* detrimental, in sepsis: the appreciation that a compensatory anti-inflammatory response phase occurs in sepsis either after or in concomitance with the classically targeted systemic inflammatory response syndrome (79) explains the unfortunate past failures but also represents an opportunity for resolution-based therapies. Promotion of 'optimal' degree of leukocyte activation and trafficking, together with the pro-phagocytic properties discussed above and shared by quite a few pro-resolving mediators, represent the ideal feature of drugs for sepsis where bacteria containment is a major goal to avoid systemic invasion that may lead to multiple organ failure (80, 81). It remains to be seen whether such an approach will be viable in clinical settings, notoriously with unfavourable odds when drug development for sepsis is embarked upon.

Possibly even more complex is the development of therapeutics to control Alzheimer's disease, aggravated by the long-term incubation of the disease and the structural changes that have occurred before symptom manifestation. Again, the complex biology of resolution can offer an option here, with the ability to phagocytose plaque components being of great importance as well as the property of moderating the activation status of the glia, if not directly modifying cell phenotype towards a pro-resolving one, as shown in the periphery (82, 83).

These are just examples, but they allow us to propose that resolution pharmacology can not only provide a new opportunity for diseases currently controlled with 'classical anti-inflammatory' strategies, but can also enable an innovative opportunity for the clinical management of complex pathologies in striking need of therapeutic options.

What would be the features of a pro-resolving drug?

In **Figure 2** we have indicated the predicted pharmacodynamics of resolution-based therapeutics. These medicines will be activating endogenous mechanisms that terminate inflammation and promote repair. Another fundamental distinction from current anti-inflammatory therapy lies in the fact that resolution-based drugs will elicit multiple actions rather than evoke a single mechanism. Thus, resolution-based drugs will have a profile truly distinct from the concept of the 'magic bullet' pursued in the last decades by drug discovery

programmes (85). The dated nature of this concept emerges from the recent appreciation that common diseases are multi-varied in their pathogenesis: patient stratification will demand the identification of personalised therapeutic strategies. In addition to engaging multiple processes, pro-resolving therapeutics are modulatory in their profile, with observed effects often within the range of 30-50% modulation. This is not a trivial point because we predict that such a bio-mechanism likely will underlie a low burden of side effects for resolution-based drugs. There are no data from human studies, but ground-breaking work from Charles Serhan's group demonstrates how LXA_4, RvD1 and other resolvin-based bioactive mediators do not hamper the host response to infection, quite the opposite. Chiefly demonstrated in bacterial infection (86), further work has corroborated this finding following viral infection (87), indicating a fundamental distinction from the most recent development for antiinflammatory therapeutics: strategies aiming at blocking the actions of tumor necrosis factor or other cytokines/cytokine receptors have been accosted to a re-emergence of *Mycobacterium tubercolosis* and other infections, due to the marked immunosuppression they cause (88). We predict that the modulatory rather than strongly inhibitory approach afforded with resolution pharmacology discussed above and depicted in Figure 2 should avoid this major clinical problem, a prediction that must be consolidated with clinical trials.

Concluding remarks

We have reasoned here on the development of resolution pharmacology as a new strategy to drug discovery programmes aiming to yield the anti-inflammatory therapeutics of the next decade. Following a fertile period of basic research in experimental inflammation and the definition of players, targets and mechanisms of Resolution, time is ripe to establish the translational potential for this research and effectively develop new drugs on the market. Our prediction is that medicines developed under the biology of Resolution and developed through the exploitation of validated pro-resolving signatures, will be different and possibly devoid of major side effects, as well as more tailored to incite protective and reparative processes within the patients themselves. Whether we are right or wrong, only time will tell (see 'Outstanding Question Box'). However not capitalizing on this new rationale and the underpinning science, might be a missed opportunity if not a true mistake detrimental to the ever increasing patient populations of Western societies affected by common debilitating pathologies.

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Figure Legends

Figure 1. **Time-phase engagement of pro-resolving mediators.**
Inflammation is a physiological response that, when effectively controlled in extent and time, leads to tissue protection and restoration of homeostasis, without causing excessive tissue damage (profile [1]). However, an exaggerated response to inflammatory stimuli can have detrimental consequences and result in substantial tissue harm, as in profile [2]. An effectively mounted inflammatory response will also imply the activation of pathways intended to safely terminate the inflammatory response by "cleaning up" the insulted tissue and promoting healing [profile 3]. However, a failure in pro-resolving pathways will extend in time the actions of pro-inflammatory mechanisms resulting in chronic inflammation and ceaseless or prolonged [profile 4] damage. When endogenous (or exogenously administered) pro-resolving molecules counter-regulate the pro-inflammatory phase, restoration of tissue structure and function is achieved more rapidly, with minimization of the potential detrimental effects [profile 5].

Figure 2. **Inflammation Pharmacology *vs.* Resolution Pharmacology.** The strategies of targeting the pro-inflammatory or the pro-resolving phase of inflammation are diametrically different. Pro-resolving molecules would deliver actions already "optimised" by nature. For example, while there are not natural COX inhibitors nor we have TNFα blocking antibodies in our blood, the processes of apoptosis and efferocytosis of neutrophils are natural physiological responses that occur several times in one's life. Classical inflammation pharmacology aims to *block or inhibit* particular mediators which, when produced in excess, are the responsible of tissue damage (e.g. TNFα, prostaglandins...). The resolution pharmacology we define here is based on a strategy that focuses on *activating or intensifying* cellular processes that participate in limiting or preventing damage, such as clearance of potentially dangerous apoptotic cells (efferocytosis). To date, a number of endogenous tissue protective/pro-resolving mediators have been identified: annexin A1, melanocortins, lipids such as lipoxin A_4, resolvins or protectins, adenosine, somatostatin or galectins to name a few. Another fundamental difference is the non-reductionist nature of pro-resolving mediators, as they typically exert broad actions instead of causing, for instance, inhibition on a selected mediator or pathway. In addition, mild-to-moderate actions characterize pro-resolving molecules: they balance the responses in order to reach equilibrium between pro- and anti-inflammatory actions. We see resolution as a way to preserving the inflammatory response by ensuring that the necessary life-saving pro-inflammatory signals are prevented from over-shooting. Side effects associated with the pro-resolving strategy itself (not considering for example off-targets effects of a particular new

molecule) are not known. We predict that by exploiting natural processes of tissue preservation and repair, pro-resolving drugs will be devoid of major side effects (see main text for reasoning on susceptibility to infection). However this prediction needs to be tested in proper clinical trials.

Figure I. Pro-resolving mediators Annexin A1 and alpha-MSH ID Cards.

Figure II. Approaches to GPCR drug discovery

Box 1. The identity card of a pro-resolving mediator.

Endogenous pro-resolving mediators have been characterized for their multiple functions that aim at the tight control – in space and time – of the inflammation response. As such, multiple cells targets, sometimes as a consequence of distinct receptor engagement but mainly through a specific pro-resolving receptor, are modulated *in an active* fashion to achieve the homeostatic goal as discussed in this Review. As such specific sets of actions are being identified as characteristics of pro-resolving mediators and pathways that allow the proposition of an identity card (**ID Card)** as presented herein.

WHAT are they? They can be lipids, short or long peptides, proteins and molecular (gases) nature.
WHO produce them? Immune cells and stromal cells (that vary in a tissue specific fashion).
WHEN do they act? These mediators effect homeostatic functions by modulating onset and resolution phases of the inflammatory response.
WHY are they produced? To ensure an effective inflammatory response enabling tight tissue protection thus limiting damage (during the onset and peak phases) and promote tissue repair and healing (in the resolving phase).
WHERE are they expressed? Often produced at the site of inflammation (local protective circuits) however can be generated in the circulation (inflammation from within).
HOW do they act? By activating specific pro-resolving receptors (on the cell surface) or molecular targets (intracellular).

Figure I below illustrate the Annexin A1 ID Card and the alpha-MSH ID Card.

Box 2. Strategies in pro-resolving GPCR drug discovery.

G-protein-coupled receptors (GPCRs) constitute the most prosperous protein families for drug discovery, with 30-50% of all approved drugs acting by targeting GPCRs. The complexity of GPCRs is determined by the diversity of external stimuli they can respond to (including lipids, amino acids, proteins, hormones, nucleotides, neurotransmitters, light, etc) and the variety of intracellular pathways they can engage, which depends in part by which particular Gα, Gβ, or Gγ proteins they are coupled to. Classical paradigms stated that a GPCR could exists in an inactive or active state, this latter happening when engaged by an agonist, which drives the activity. However, the current understanding suggests that the activity is an intrinsic property to the receptor itself, but kept silent by intramolecular constraints. The activity in quality and quantity) will depend on which molecular constraints are relieved by a particular agonist. This notion better explains the existence of GPCR features like constitutive activity, biased agonism or allosteric modulation. These features, explained next, are only expected to increase the number of novel drugs targeting the already largest drug target protein family. Currently, the three main approaches to GPCR drug discovery (see Figure II below) include:

I) ***Orthosteric agonists*** are molecules that bind to a receptor and elicit a cellular response. Orthosteric refers to the site where the endogenous ligand binds. By contrast, an ***antagonist*** is a molecule that binds to the receptor without eliciting a cellular response, while an ***inverse agonist*** will elicit a cellular response that is the opposite to that elicited by the agonist.

II) ***Positive allosteric modulators*** (PAMs) are drugs that, binding to a site topographically distinct from the site where the natural ligand binds, enhances the activity of the natural ligand, without eliciting any response on its own. Benzodiacepines and barbiturates are examples of PAMs at the $GABA_A$ receptor. This term should not be confused with ***allosteric agonist***, which are molecules that elicit a response but binding to a site distinct to the natural ligand binding site (orthosteric site). ***Bitopic drugs*** are molecules that can interact simultaneously with orthosteric and allosteric sites.

III) ***Biased agonist*** are drugs that can only stabilize a subset of possible active conformations for that receptor and hence activate selective pathways from the signaling repertoire of that particular receptor. This new mode of action brings up the concept of functional selectivity, making possible the design of molecules that selectively activate the therapeutically relevant pathways and not those associated with side effects. Again, “mixed up” options can exist such as ***allosteric biased agonist***, which will imply that the drug binds to a topographically different pocket to the one used by the agonist.

Outstanding Questions Box.

The major questions that the field of resolution of inflammation will be soon facing in the context of development of resolution pharmacology are the following ones:

- Can a pro-resolving based drug effectively control an on-going chronic pathology?

Most of the preclinical work has been conducted in settings of acute inflammation, however these new drugs may provide a novel therapeutic opportunity in chronic settings.

- Would a resolution-based therapeutic strategy effectively be devoid of side effects?

A hallmark of the philosophy behind resolution is the activation of endogenous tissue-protective pathways, predicting less toxicity. However, this must be proven.

- Can we use resolution players and targets to stratify patients in relation to the pathotype of specific diseases and/or responsiveness to therapeutic treatment?

Development of novel analytical protocols can allow monitoring the expression patters of players of resolution. This can represent a very novel tool to define the patient status and predict responsiveness.

Table 1. Resolution Pharmacology: what's going in the translational arena.

Compound	Health condition studied	Trial number and/or publication	Notes
Melanocortins			
Tetracosactide	Gouty arthritis	EUCTR2011-000069-11-ES	Efficacy and safety
Synacthen Depot®	Idiopathic membranous nephropathy	ISRCTN70791258	Phase II
H.P. Acthar® Gel	Systemic lupus erythematosus	NCT01939132	Phase IV
ACTH	Atopic dermatitis, aged skin	JPRN-UMIN000012511	Phase II
AP214	Post-surgical AKI	EUCTR2010-022630-92-DK	αMSH analog; Phase II
ABT-719 (AP214)	AKI	NCT01777165	αMSH analog; Phase II
Afamelanotide [$]	Erythropoietic protoporphyria	NCT01605136	αMSH analog; Phase III
Lipoxin and Resolvins			
Pioglitazone	Type 2 Diabetes Mellitus	NCT01040819	LXA_4 production; Phase IV
RX-10045	Dry eye syndrome	NCT00799552	RvE1 analog; Phase II
BLXA4-ME	Gingival inflammation	NCT02342691	FPR2/ALX agonist; Phase I/II
Somatostatin			
Somatostatin	Acute severe upper gastrointestinal bleeding	NCT00152399	Phase II
Long-acting release (Sandostatin-LAR)	Polycystic kidney	NCT00309283	Phase III; Results in PMID: 23972263
FX125L	Asthma	EUCTR2014-002052-84-GB	SSTR2 agonist; Phase IIA
FX125L	COPD	EUCTR2011-005036-26-GB	Phase IIA
Adenosine			
CF101	Rheumatoid arthritis	NCT01034306 and CTRI/2012/12/003205	ADORA3 agonist; Phase II
CF101	Uveitis	NCT01905124	ADORA3 agonist; Phase II
BVT-115959	Diabetic neuropathy	NCT00452777	ADORA2A agonist; Phase II
Regadenoson (CVT-3146)	Asthma	NCT00862641	ADORA2A agonist; Phase IV
Sonedenoson (MRE-0094)	Diabetic foot ulcers	NCT00318214, NCT00312364	ADORA2A agonist; Phase II
UK-432,097	COPD	NCT00430300	ADORA2A agonist; Phase II
Cannabinoids			
Dronabinol (THC)	Irritable bowel syndrome	NCT01786109	CB2 agonist; Phase II; Results in PMID: 21803011
Resunab (ajulemic acid)	Cystic fibrosis, scleroderma	[$$]	CB2 agonist; Phase I
PF-04457845	Osteoarthritis	NCT00981357	Fatty acid amide hydrolase inhibitor; Phase II
JNJ-42165279	Healthy volunteers	NCT01964651	Fatty acid amide hydrolase inhibitor; Phase I
Omega-3			
Omega-3 fortified drink	Atherosclerosis	NCT00886704	Results in PMID:

			20420756
EPA/DHA	Non-alcoholic fatty liver disease	WELCOME Study	Results in PMID: 25043514
DHA	Primary sclerosing cholangitis	NCT00325013	Phase I
Glucocorticoids	Asthma patients under glucocorticoid treatment	NCT01761630	Observational; Expression of LXA_4, AnxA1, SAA and FPR2/ALX
Fish oil-based lipid emulsion	Healthy volunteers under endotoxin inhalation	DRKS00006131	Phase II; Results in PMID: 25962383
Carbon monoxide			
BI 113608	Healthy volunteers	NCT01540825	Phase I
Nitric Oxide			
Beet root (nitrate supplementation)	Hypertension	NCT01236872	Phase I
Beet root (nitrate supplementation)	Hypertension	NCT01405898	Phase II; Results in PMID: 25421976
Hydrogen Sulphide			
N-Acetyl-Cysteine	Chronic kidney disease	NCT01232257	Phase III

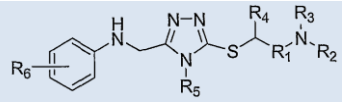
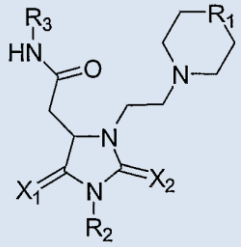
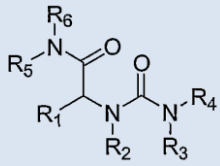
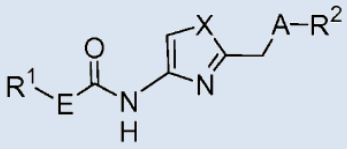
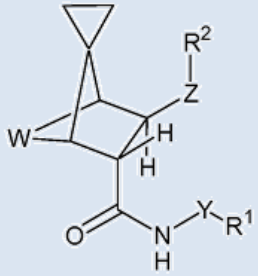
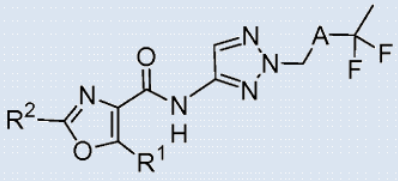
A non-exhaustive list of registered trials with a rationale based on players and targets of the Resolution of Inflammation, hence with a potential to establish Resolution Pharmacology. The variety of indications can be noted, together with the distinct approaches taken to exploit resolution pathways, as discussed in the main text. In some cases publications (PMID) resulted from the clinical investigation are reported. More trials can be found at http://apps.who.int/trialsearch/.

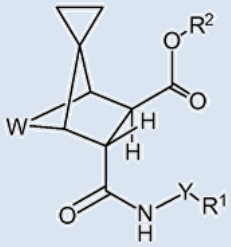
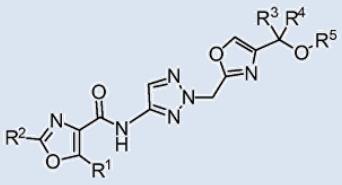
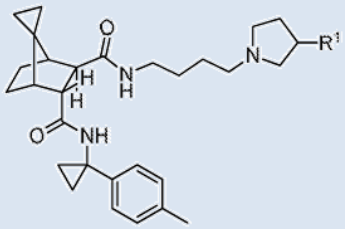
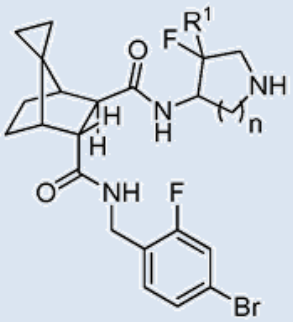
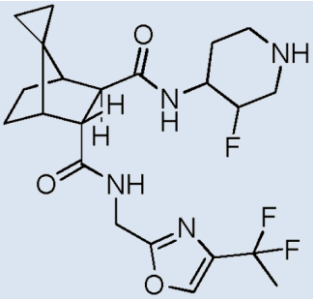
Abbreviations: ADORA, adenosine receptor A; AKI, acute kidney injury; CB; cannabinoid; COPD, Chronic Obstructive Pulmonary Disease; EMA, European Medicine Agency; FPR2/ALX, formylpeptide receptor type 2, lipoxin A_4 receptor; SAA, Serum Amyloid protein A; SSTR2, somatostatin receptor type 2;

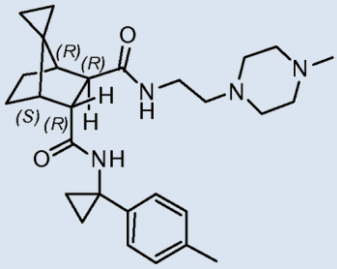
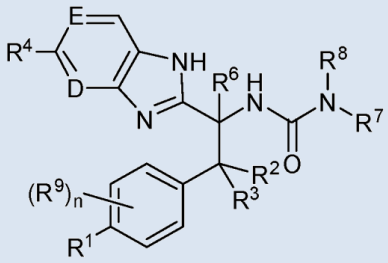
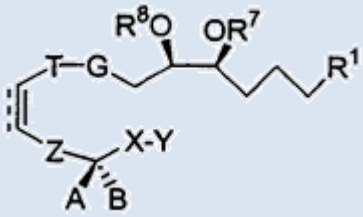
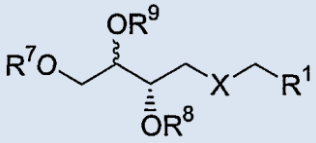
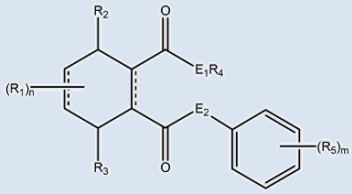
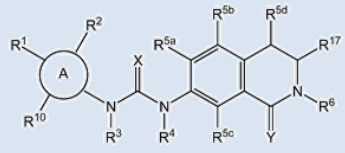
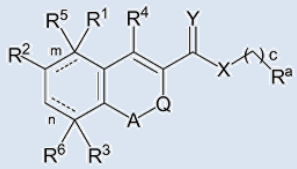
[$] Approved by the EMA; www.clinuvel.com/en/investors/news-publications/announcements/2014-announcements/scenesse®-attains-historic-breakthrough-european-marketing-authorisation

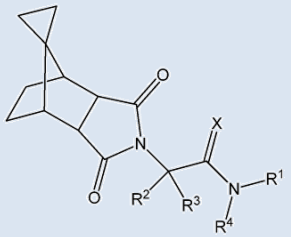
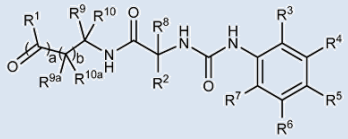
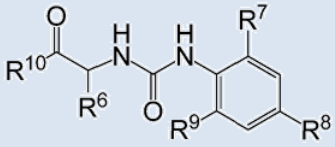
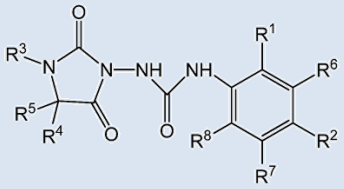
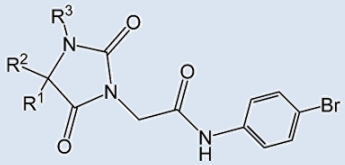
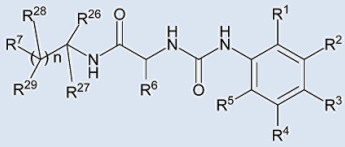
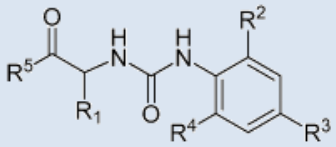
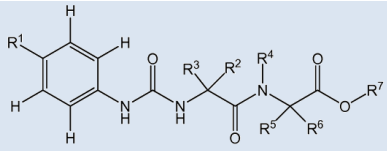
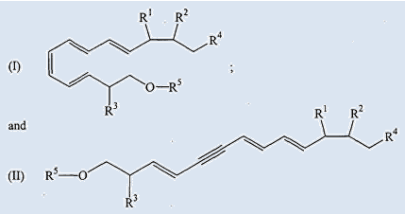
[$$] www.corbuspharma.com/product-pipeline/resunab

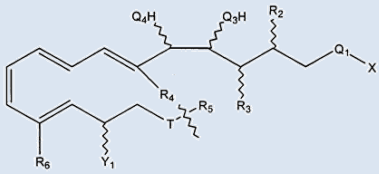
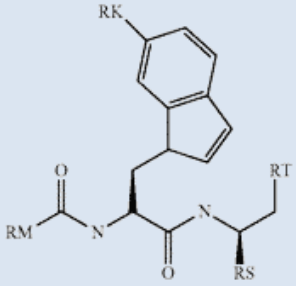
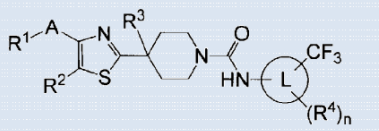
Table 2. Small molecule agonists at the FPR2/ALX receptor: an opportunity for innovative pro-resolving drugs?

Patent Family	Molecule Structure	Comments and Notes	1st claimed formula/backbone
Acadia Pharmaceuticals, Inc.			
WO 2005047899	Use of the lipoxin receptor, fprl1, as a tool for identifying compounds effective in the treatment of pain and inflammation	Priority Nov 7, 2003 Treating inflammation and associated pain, including cardiovascular diseases and chemical injury.	(I)  (II)  (III) 
Actelion Pharmaceuticals, Ltd.			
a)WO 2009077990 b)WO 2010143158	a) Aminotriazole derivatives b) Oxazole and thiazole derivatives	a) Priority Dec 18, 2007 Inflammatory diseases, obstructive airway diseases, allergic conditions, HIV-mediated retroviral infections, cardiovascular disorders, neuroinflammation, neurological disorders, pain, prion-mediated diseases and amyloid-mediated disorders; and for the modulation of immune responses. b) Priority Jun 12, 2009 (Indications as above)	
WO 2010134014	Bridged spiro [2.4] heptane derivatives	Priority May 18, 2009 (Indications as above)	
WO 2010143116	Fluorinated Aminotriazole Derivatives	Priority Jun 9, 2009 (Indications as above)	

WO 2012066488	Bridged spiro [2,4]heptane ester derivatives	Priority Nov 17, 2010 (Indications as above)	
WO 2012077049	Oxazolyl-methylether derivatives	Priority Dec 7, 2010 (Indications as above)	
WO 2013171687	1-(p-tolyl)cyclopropyl substituted bridged spiro[2.4]heptane derivatives	Priority May 16, 2012 (Indications as above)	
WO 2013171694	Fluorinated bridged spiro[2.4]heptane	Priority May 16, 2012 (Indications as above)	
WO 2014206966	Difluoroethyl-oxazole substituted bridged spiro[2.4]heptane derivatives	Priority Jun 25, 2013 (Indications as above)	

WO 2015007830	Piperazine substituted bridged spiro[2.4]heptane derivatives	Priority Jul 18, 2013 (Indications as above)	
WO 2015019325	Benzimidazolyl-methyl urea derivatives	Priority Aug 9, 2013 (Indications as above)	
Alcon Universal, Ltd. / Alcon Research, Ltd.			
WO 2001034144	Lipoxin A_4 and its analogs	Priority Nov 9, 1999 Dry eye & post-surgical ocular trauma	
WO 2006052950	5,6,7-trihydroxyheptanoic acid and analogs	Priority Nov 9, 2004 Posterior segment ocular disease (diabetic retinopathy, wet AMD, retinal microvasculopathy, and retinal edema) or a cellular hyperproliferative disorder (vascular restenosis secondary to a percutaneous transluminal coronary angioplasty procedure). *Background assignee appears to be Novartis AG*	
Allergan, Inc.			
WO 2011163502	Derivatives of cycloalkyl- and cycloalkenyl-1,2-dicarboxylic acid	Priority Jun 24, 2010 Ocular diseases	
WO 2012109544	Novel 1-(1-Oxo-1,2,3,4-tetrahydroisoquinolin-7-yl)urea derivatives	Priority Feb 11, 2011 Ocular diseases	
WO 2012125305	Dihydronaphthalene and naphthalene derivatives	Priority Mar 17, 2011 Ocular diseases, vascular diseases, infectious disorders, CNS diseases, and more.	

WO 2013009543	Polycyclic pyrrolidine-2,5-dione derivatives	Priority Jul 11, 2011 (Indications as above)	
WO 2013062947	Amide derivatives of n-urea substituted amino acids	Priority Oct 26, 2011 (Indications as above)	
WO 2013070600	Aryl urea derivatives	Priority Nov 10, 2011 (Indications as above)	
WO 2013071203	2,5-dioxoimidazolidin-1-yl-3-phenylurea derivatives	Priority Nov 10, 2011 (Indications as above)	
WO 2013122953	Imidazolidine-2,4-dione derivatives	Priority Feb 16, 2012. (Indications as above)	
WO 2013158597	(2-ureidoacetamido) alkyl derivatives	Priority Apr 16, 2012 (Indications as above)	
a) WO 2014138037 **b)** WO 2014138046	Use of agonists of formyl peptide receptor 2	a) Priority Mar 6, 2013 Ocular indications b) Priority Mar 16, 2013. Dermal indications.	
WO 2015009545	N-urea substituted amino acids	Priority Jul 16, 2013 Ocular diseases	
Bayer Schering Pharma AG			
a) WO 2003040080 **b)** WO 2008022807	Lipoxin A_4 analogs	a) Priority Nov 6, 2001 Inflammatory or autoimmune disorders (RA, OA, lupus, AD, vascular, and more) b) Priority Aug 23, 2006 IBD, colitis	
Brigham and Women's Hospital Boston			

WO 2000055109	Lipoxin compounds	Priority Mar 18,1999 Disease or condition associated with polymorphonuclear leukocyte inflammation	
WO2002070068	Lipoxin analogs	Priority Mar 2, 2001	LXA_4 or 15-R/S-methyl-LXA_4 or 5-epi-16-(para-fluoro)-phenoxy-lipoxin
Chang Gung University			
US20150099691	N—(N-aroyl-L-tryptophanyl)-D-phenylalanine methyl esters	Priority Oct 9, 2013 Neutrophil inflammatory disorders: lung injury, COPD, ARDS, asthma, IRI, arthritis and septicemia	
ONO Pharmaceutical Co., Ltd.			
WO 2015005305	-N- [4-(trifluoromethyl) phenyl] -1-piperidinecarboxamide derivatives	Priority Jul 9, 2013 FPT2/ALX related diseases including autoimmune diseases, asthma, pulmonary fibrosis, atopic dermatitis, ischemia-reperfusion injury, myocardial infarction or Alzheimer's disease.	
University of Louisiana State			
WO 2009058815	Lipoxin A_4 and its analogs	Priority Oct 29, 2007 Ocular disease	Docosahexaenoic acid and neuroprotectin D1 specifically named

Search criteria: Full text search performed “FPR2”, “ALXR” and “FPRL”, sub-search of IPC code A61 [Human Necessities: Medical or Veterinary Science; Hygiene], primarily on https://www.patbase.com. Searches carried out on 15/05/2015.

Figure 1

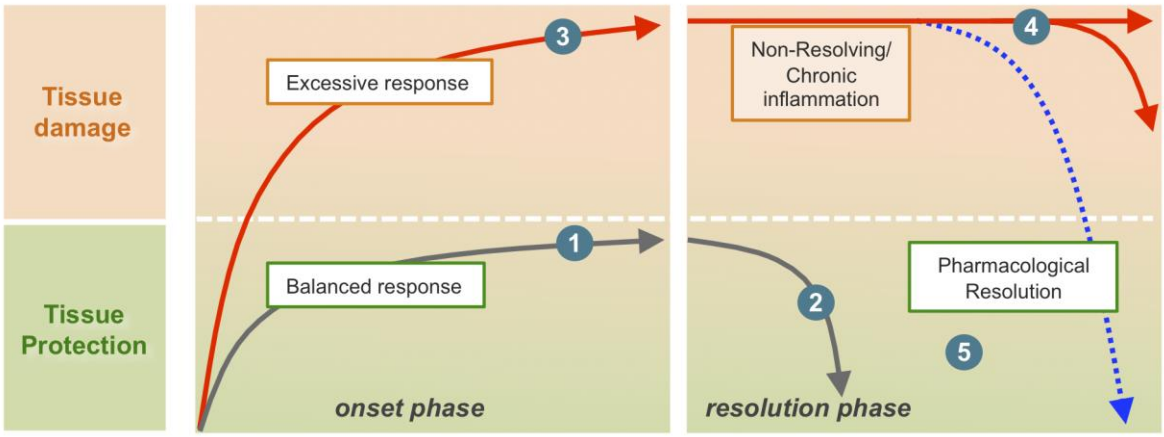
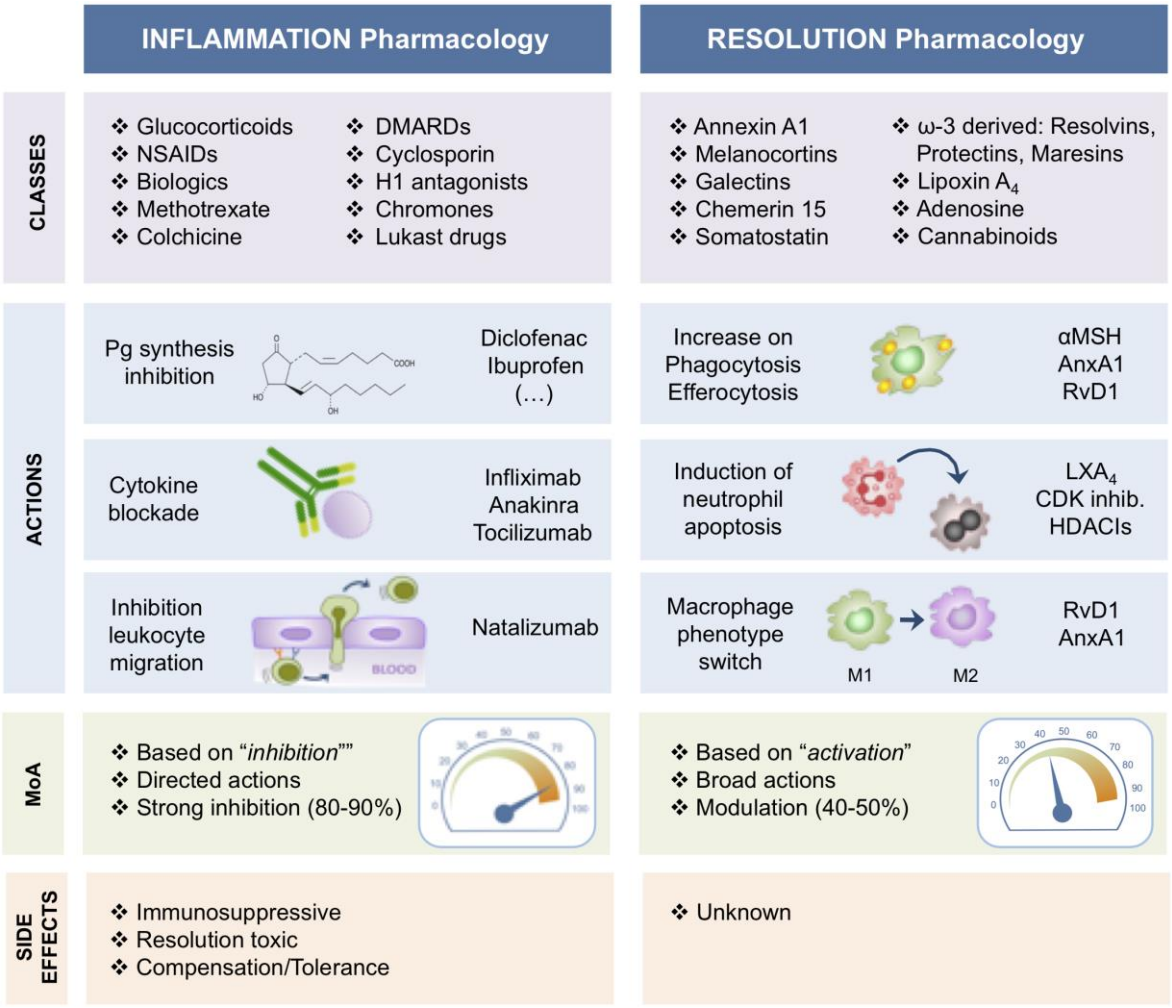

Tissue damage
Tissue Protection
Excessive response
Balanced response
onset phase
Non-Resolving/ Chronic inflammation
Pharmacological Resolution
resolution phase
1
2
3
4
5

Figure 2


INFLAMMATION Pharmacology
RESOLUTION Pharmacology
CLASSES
❖ Glucocorticoids
❖ NSAIDs
❖ Biologics
❖ Methotrexate
❖ Colchicine
❖ DMARDs
❖ Cyclosporin
❖ H1 antagonists
❖ Chromones
❖ Lukast drugs
❖ Annexin A1
❖ Melanocortins
❖ Galectins
❖ Chemerin 15
❖ Somatostatin
❖ ω-3 derived: Resolvins, Protectins, Maresins
❖ Lipoxin A4
❖ Adenosine
❖ Cannabinoids
ACTIONS
Pg synthesis inhibition
Diclofenac
Ibuprofen
(…)
Increase on Phagocytosis Efferocytosis
αMSH
AnxA1
RvD1
Cytokine blockade
Infliximab
Anakinra
Tocilizumab
Induction of neutrophil apoptosis
LXA4
CDK inhib.
HDACIs
Inhibition leukocyte migration
BLOOD
Natalizumab
Macrophage phenotype switch
M1
M2
RvD1
AnxA1
MoA
❖ Based on "inhibition""
❖ Directed actions
❖ Strong inhibition (80-90%)
❖ Based on "activation"
❖ Broad actions
❖ Modulation (40-50%)
SIDE EFFECTS
❖ Immunosuppressive
❖ Resolution toxic
❖ Compensation/Tolerance
❖ Unknown