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DR. JOAKIM S DAHLIN (Orcid ID : 0000-0003-3007-9875)
PROF. MARCUS MAURER (Orcid ID : 0000-0002-4121-481X)
DR. DEAN D. METCALFE (Orcid ID : 0000-0002-7781-8260)
PROF. GUNNAR PEJLER (Orcid ID : 0000-0002-6779-391X)
PROF. GUNNAR NILSSON (Orcid ID : 0000-0001-6795-5512)

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The Ingenious Mast Cell: Contemporary insights into mast cell behavior and function

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Joakim S. Dahlin[1], Marcus Maurer[2], Dean D. Metcalfe[3], Gunnar Pejler[4,5], Ronit Sagi-Eisenberg[6], Gunnar Nilsson[1,7]

[1]Division of Immunology and Allergy, Department of Medicine, Karolinska Institutet, and Karolinska University Hospital, Stockholm, Sweden; [2]Dermatological Allergology, Allergie-Centrum-Charité, Department of Dermatology and Allergy, Charité—Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany;[3]Mast Cell Biology Section, Laboratory of Allergic Diseases, NIAID, NIH, Bethesda, MD, USA; [4]Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden, [5]Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences, Uppsala, Sweden; [6]Dept. of Cell and Developmental Biology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; [7]Department of Medical Sciences, Uppsala University, Uppsala, Sweden.

ORCID numbers:
JSD 0000-0003-3007-9875
MM 0000-0002-4121-481X
DDM 0000-0002-7781-8260

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GP 0000-0002-6779-391X
RSE0000-0002-1990-8831
GN 0000-0001-6795-5512

Correspondence to: Gunnar Nilsson, division of immunology and allergy, department of medicine, Karolinska Institutet, 171 76 Stockholm, Sweden. E-mail:gunnar.p.nilsson@ki.se

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Abbreviations:

BTK	Bruton's tyrosine kinase
CTMC	Connective tissue mast cells
CU	Chronic urticaria
CSU	Chronic spontaneous urticaria
HαT	Hereditary alpha tryptasemia
MC	Mast cell
MCp	Mast cell progenitor
MCAS	Mast cell activation syndrome

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MMAS	Monoclonal mast cell activation syndrome
MMC	Mucosal mast cells
PMD	Piecemeal degranulation
SCF	Stem cell factor
SG	Secretory granule
SYK	Spleen associated tyrosine kinase

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Abstract

Mast cells are (in)famous for their role in allergic diseases, but the physiological and pathophysiological roles of this ingenious cell are still not fully understood. Mast cells are important for homeostasis and surveillance of the human system, recognizing both endogenous and exogenous agents, which induce release of a variety of mediators acting on both immune and non-immune cells, including nerve cells, fibroblasts, endothelial cells, smooth muscle cells and epithelial cells. During recent years, clinical and experimental studies on human mast cells as well as experiments using animal models have resulted in many discoveries that help decipher the function of mast cells in health and disease. In this review we focus particularly on new insights into mast cell biology, with a focus on mast cell development, recruitment, heterogeneity and reactivity. We also highlight the development in our understanding of mast cell driven-diseases and discuss the development of novel strategies to treat such conditions.

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Introduction

Although hematopoietic stem cells produce mast cell (MC) progenitors, mature MCs are normally absent in blood while found in almost all tissues as highly granulated cells. The stem cell factor (SCF) receptor, KIT, is one of the most critical receptors on mature mast cells, as a reduction in KIT signaling leads to mast cell deficiency. Yet, signaling through the receptor is redundant for early development of mast cell progenitors in peripheral blood[1].MCs are equipped with a great number of receptors enabling them to sense and react to a diversity of stimuli [2]. The most studied receptor that causes MC activation is the high-affinity IgE receptor, FcεRI. Another receptor that has received particular attention during recent years is the Mas-related G protein-coupled receptor X2 (MRGPRX2), a G-protein coupled receptor recognizing a variety of pharmacologic agents (tubocurarine, atracurium, icatibant, ciprofloxacin) causing pseudo allergic reactions[3,4]. MRGPRX2 also serves as receptor for substance P, components of insect venom, antimicrobial peptides, secreted eosinophil products and other cationic peptides[5,6]. Mast cell expression of MRGPRX2 has been associated to neurogenic inflammation, pain and itch [7-9], and also to contribute to the development of allergic inflammation [10]. Other receptors that induce IgE-independent MC activation include the IL-33 receptor, which is important for MCs to recognize cell injury and trauma[11] as well as regulating IgE-mediated responses[12-14], and pattern recognition receptors (PRRs) sensing "danger " signals, including microbes[15].When MCs sense an endogenous or exogenous agent through binding to one of their many activating receptors, they react by releasing mediators through three major pathways (Figure 1). The most rapid response is exocytosis of secretory granules (SGs) and the release of preformed mediators such as histamine, proteases and heparin[16]. This is accompanied by the *de novo* biosynthesis of lipid mediators, predominantly eicosanoids such as prostaglandin D_2 (PGD_2), the cys-leukotrienes LTC_4, LTD_4 and LTE_4, and also other eicosanoids like thromboxane A_2 (TXA_2) and 15-HETE[17], as well as other lipid mediators such as platelet activating factor (PAF) and sphingosine-1-phosphate (S1P) [18]. In addition, MCs also have the capacity to synthesize a number of cytokines, chemokines, growth factors and interferons[19]. Notably, release of *de novo*-synthesized mediators can take place without preceding degranulation (Figure 2)[20].Examples of such are the effect of IL-33 that induces secretion of leukotrienes and cytokines e.g.[21,22] , CD30 inducing release of only cytokines and chemokines [23], and certain viruses, e.g., respiratory syncytial virus, inducing secretion of type I interferons and chemokines [15,24] (Figure 2). Thus, MCs can be activated and produce lipid

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mediators and/or cytokines in the absence of detectable degranulation (by histology or measurement of granule mediators)[25]. Finally, MCs also secrete extracellular vesicles including exosomes. MC exosomes can transfer proteins, enzymes, RNA and miRNA that can be taken up by other cells, either proximal to the secreting MC or located at distant sites[26-29] (www.exocarta.org) (Figure 1). Notably, patients with systemic mastocytosis have increased levels of exosomes with a MC signature including constitutively activated KIT, enabling transfer of mutant proteins to other cells[30].

Given the broad distribution of MCs and their multifunctional role, the variety of mediators released, the symptoms associated with mast cells mediators (Figure 3), they have been implicated in many diseases beyond allergy[31-33]. For example, recent reviews highlight the role of MCs in cardiovascular diseases[34], cancer[35], airway diseases[36], as well as in viral, bacterial and fungal infections[37-39]. Even if MCs commonly are discussed in the context of disease, it is important to remember that they also have a role in homeostasis, the initiation of acute inflammation[40] and in the protection against danger, whether it comes from the outside (venoms, pathogens, etc.) or from within the body (cell injury etc.)[41].

In this review we highlight some of the most recent findings regarding MC origin and development, recruitment, heterogeneity and reactivity, MC disease, new therapeutic possibilities, and animal models to study MC biology.

The origin of mast cells

Hematopoietic cells arise in several temporally distinct waves during prenatal development. The first MCs originate from primitive erythromyeloid progenitors in the extraembryonic yolk sac[42-45]. A second wave of MCs appears together with the first definitive hematopoietic progenitors. The first two waves mainly contribute to connective tissue MCs (CTMC) and serosal-type MCs[43]. The third hematopoietic wave originates from the aorta-gonado-mesonephros region. Cells formed during this wave produce hematopoietic stem cells that exhibit MC-forming potential[43]. It was for a long time assumed that MCs develop from hematopoietic stem cells in the bone marrow[46]. However, it is now clear that bone marrow hematopoietic stem cells produce a fraction, but not all,

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MCs. Experiments also show that bone marrow-derived MCs mainly replenish the mucosal MC population postnatally[43]. However, there is a redundancy in the potential of the waves as bone marrow-derived MCs can reconstitute depleted skin-resident (connective tissue-type) MCs and populate the skin following inflammation[47].

From hematopoietic trees to differentiation landscapes

The classic tree-like model that describes hematopoiesis assumes a stepwise loss of lineage potential with differentiation from hematopoietic stem cells to lineage-committed progenitors (Figure 4A). However, assignment of the MC differentiation trajectory to either the granulocyte-monocyte or the megakaryocyte-erythrocyte branch is a controversial topic. Studies supporting the idea that MCs belong to either branch have been presented[48-53]. On a different angle, other investigations propose that MCs branch off early – close to hematopoietic stem cells – and differentiate along a unique trajectory[54,55].

Advances in high-throughput single-cell RNA sequencing and computational biology are now delineating the MC differentiation trajectory by suggesting a revision of the hematopoietic model[56]. The idea to reconstruct hematopoietic differentiation with single-cell transcriptomics is based on capturing a snapshot of single differentiating progenitors[57]. Such analysis reveals that differentiating cells traverse a continuous landscape of states, from multi- and bipotent progenitors to lineage committed cells (Figure 4B). A single-cell transcriptional landscape of bone marrow hematopoiesis in mouse shows that the MC developmental trajectory is positioned adjacent to the erythrocyte and basophil trajectories[51,58]. The MC and basophil trajectories are particularly close to each other[58,59], which indicates the presence of a bipotent basophil-MC progenitor population, a finding that is recapitulated in single-cell fate assay experiments [49,58-60]. Cell fate assays of mouse bone marrow progenitors also verified that basophil/MC differentiation is closely coupled with erythropoiesis[51]. The combination of single-cell transcriptomics and lineage tracing provides even deeper insights into hematopoiesis with focus on MC differentiation. Tracing individually barcoded mouse hematopoietic progenitor cells during *in vitro* culture confirms a coupling between MC and basophil fates as well as between MC and erythrocyte fates[61].

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In human, single-cell transcriptomics analysis of bone marrow cells indicate that basophils and mast cells arise from a common bipotent progenitor[59]. However, cell fate assays are yet to verify the existence of such progenitor. A link between basophil/MC progenitors and erythrocyte progenitors has also been proposed in human hematopoiesis[62-65]. In analogy to lineage tracing experiment in mouse, tracking a somatic mutation in human hematopoiesis could potentially decipher the relationship between basophil and mast cell differentiation. The cellular distribution of KIT D816V mutation, a mutation present in systemic mastocytosis patients causing constitutive KIT signaling, has been studied for this purpose. No evidence supporting a close developmental relationship between mast cells and basophils has been observed using this approach[66]. In fact, the results based on charting the cellular distribution of the KIT D816V mutation seem to suggest that basophils and mast cells are distantly related. However, such analysis rests on a disputable assumption; presence or absence of the somatic KIT D816V mutation in a given progenitor does not influence the cell's fate potential. Specifically,it is possible that the acquired D816V mutation primes a multi- or bipotent progenitor towards the mast cell fate, given that KIT signaling promotes mast cell differentiation[67]. This latter idea supports a close association between basophil and mast cell differentiation in health and explains the seemingly contradictory results in disease.

In summary, single-cell RNA sequencing has revolutionized our understanding of MC differentiation. The data reveals an overall landscape of hematopoiesis and an association between the MC, erythrocyte and basophil granulocyte differentiation trajectories. The landscape model of hematopoiesis unifies old and new theories of the MC differentiation field and constitutes a foundation for future research.

Mast cell heterogeneity – how different are they from each other?

MC heterogeneity was first described in the mid-60s by differences in histochemical staining features, and the concept of connective tissue mast cells (CTMCs) and mucosal mast cells (MMCs) was born[68,69]. In humans, different MC subpopulations have been defined by their protease content; those that express tryptase (MC_T), tryptase and chymase (MC_{TC}) and chymase only (MC_C)[70,71]. The latter was, for a long time, controversial, and the presence of chymase-only positive MCs has remained ignored and virtually unstudied. The two distinct MC subpopulations,

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MC_T and $MC_{TC,}$ as well as the MMC and CTMC, differ in their localization, mediator content and responsiveness to secretagogues[72].

Besides dividing MCs into subpopulations based on their localization or protease content, there are also definitions of MCs being either constitutive or inducible, inflammatory (iMC), or pro- or anti-tumorigenic (MC1 or MC2)[35,73-75]. While the division into constitutive and inducible subpopulations might be related to the prenatal origin of the MC (see above), the latter is probably dependent on changes in the microenvironment during an inflammation. It is clear that MCs in different organs differ in their receptor and mediator expression, but also within a single tissue there is a considerable heterogeneity, e.g., among human lung MC_T[76]. Furthermore, the gene/protein expression within this MC_T subpopulation changes during inflammatory responses[75,77]. A question to address is if these changes relate to different subtypes or a plasticity within the MC population.

The origin and development of different MC subpopulations have been enigmatic, but one clue was described recently as mentioned above[45]. For hematopoiesis in the adult, the question has been if there are several different MC progenitor populations in circulation, or if there is one progenitor population that has the capacity to differentiate and mature into any of the MC subtypes. In other words, is the heterogeneity driven by locally produced factors or is it driven by the recruitment of different types of designated progenitors? In a study where this question was addressed, the results suggested the existence of a common MC progenitor that gives rise to all MC subpopulations[78]. However, single cell RNA sequencing and single cell cultures will likely provide further proof and insights into this process.

The separation of MCs into different subpopulations based on their protease content or histochemical properties is rather simplistic. MCs show a great plasticity, and detailed analysis of protease expression in human lung MCs demonstrate a gradient in the expression of the different proteases in these cells. Transcriptome comparisons of MCs from different mouse tissues with other immune cells revealed that MCs form a distinct population well separated from all other immune cells[79]. Within the MC population there was a substantial heterogeneity across tissue. Similarly, proteome analysis of human skin and fat MCs confirmed the unique identity of MCs among immune lineages, including the granulocytes (neutrophils, eosinophils and basophils)[80]. In this study, there were few differences in the proteome between skin and fat mast cells, raising

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questions on the impact of the microenvironment for MC heterogeneity [80]. Analysis of single-cell RNA sequencing data of mast cells from human tissues will further decipher MC heterogeneity and plasticity, andmight provide a clearer view about differences among MCs in different tisues, and also within the same tissue.

Mechanisms behind directions that mast cells take during their life cycle

Homing and migration

MC progenitors circulating in the blood, in humans defined as $CD34^+$, KIT^+, $Fc\varepsilon RI^+$ cells[81], enter a specific tissue where they complete their maturation under the influence of locally produced factors. Surprisingly, the homing mechanisms for MC progenitors are still mostly unknown, except that it is a tissue specific process[82-84]. In addition to their basal homing, MC numbers clearly increase at sites of inflammation, such as in allergic rhinitis and asthma. This increase in MC numbers is likely a consequence of both increased proliferation, migration and survival, as well as maturation of their progenitors recruited in response to chemoattractants[85,86]. The fact that some chemokine receptors, such as CCR1, CXCR2 and CXCR4, are expressed on both progenitors and mature MCs suggests that MC accumulation at sites of inflammation may also involve relocalization of mature MCs within a specific tissue[84-89].

To migrate or to degranulate, that is the question

To avoid loss of their munitions before reaching their final destination at inflamed sites, mature MCs need to be regulated to migrate, but not yet degranulate [90](Figure 5). This is a challenging requirement as some of the MC chemokines induce degranulation of basophils[91]and synergize with secretagogues to potentiate MC degranulation[92,93]. Thus, it was proposed that chemokines may either elicit distinct signals in MCs, as opposed to basophils, or that MC SGs are linked to the cell cytoskeleton differently from the basophil granules[90]. Marked differences observed between the actin skeleton in migrating *versus* secreting MCs support the latter possibility[94]. Analysis of actin rearrangements following chemokine stimulation of MCs revealed an accumulation of pericentral actin clusters that prevent cell flattening and converge the SGs in the cell center[94]. By contrast, reduction in the actin mesh density characterizes the secretory actin phenotype. Thus, the

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migratory actin phenotype immobilizes the secretory granules by trapping them in the cell center, whereas the secretory actin phenotype supports mobility and exocytosis. Diaphanous-related formin, mDia1, appears to be key player in these actin rearrangements[94] (Figure 5).

Kiss-and-run, full or partial exocytosis? The next question is how to degranulate

As MCs reach their destination and encounter a secretagogue, the secretory actin phenotype overrides the migratory actin phenotype, relieving the secretory constraints[94]. MCs then enter a state in which they can undergo exocytosis (Figure 5). This can take place in three different ways: i) individual SGs move to the plasma membrane where they dock and fuse with the membrane and release their full content; ii) by compound exocytosis where SGs fuse with each other, forming a channel through which they release their content; or iii) "kiss-and-run exocytosis" where SGs only transiently fuse with the membrane and release only part of their content[95]. Choosing the mode of exocytosis bears physiological significance, as the distinct features and dynamics of the different exocytic modes are associated with unique physiological responses[96]. This notion was nicely illustrated in a recent study that investigated the MCs' response to innate stimulation (e.g., by the innate receptor MRGPRX2).Human MCs quickly mobilize discrete SGs toward the plasma membrane, resulting in very rapid secretion of individualSGs; by full or possibly also by kiss and run exocytosis[96]. By contrast, activation of MCs by antibody-dependent adaptive stimuli (e.g., IgE+Ag), resulted in the much slower exteriorization of groups of granules formed by the prior intracellular fusion of individual SGs, resulting in compound exocytosis[96]. Intriguingly, the distinct modes of exocytosis were linked with distinct physiological features. Thus, whereas the MRGPRX2-mediated trigger is brief and cause local inflammatory reactions, the IgE-stimulated reactions are more substantial and also lasted for a longer period of time[96].

Ultrastructural studies of MCs have implicated an additional mode of secretion, which is independent of SG exocytosis[97]. The latter, termed piecemeal degranulation (PMD), involves vesicle budding from the SGs and their transport and fusion with the plasma membrane. PMD has been associated mainly with MC activation under inflammatory conditions, including MC activation in pediatric cholelithiasis and biliary dyskinesia[98],functional dyspepsia[99] and cancer [100].PMD could in principle lead to the selective release of some SG mediators, such as reported for secretion of serotonin without histamine from rat MCs [101,102]. However, the precise mechanism of such mediator segregation awaits further investigation. At the molecular level, two factors have

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been identified as critical for defining the degranulation pattern. The first is the enzyme IKKβ, whose activity is required for the phosphorylation of the SNARE protein SNAP23, which is essential for SG fusion during compound exocytosis[95,96,103]. The second is the small GTPase Rab5, which plays a role in SG fusion during both their biogenesis[104] and compound exocytosis[105]. MCs may or may not release newly synthesized mediators, including lipid mediators, chemokines and cytokines. Whether or not such release takes place is dependent on the stimulus type and may occur with or without prior release of preformed mediators[20]. Thus, unlike the regulation of degranulation that takes place at the level of the exocytic process, regulation of cytokine and chemokine release occurs at their transcriptional level, whereas secretion utilizes the constitutive secretory and endocytic pathways[106]. Mediators derived from arachidonic acid are immediately released, however, how precisely lipid mediators that are synthesized intracellularly are released is poorly understood[20]. Finally, MCs release exosomes, whose content is dictated by the type of trigger[107].

In conclusion, the underlying mechanisms that regulate MCs during their life cycle are only beginning to be clarified. They include mechanisms that ensure MC homing to destined tissues, recruitment to inflammatory areas, prevention of premature secretion, selection of the mode of exocytosis, and which lipid mediators, cytokines or chemokines to synthesize de novo. Future studies will shed light on these mechanisms and the molecular entities involved.

Mast Cells in Diseases

MCs are involved in the initiation and perpetuation of a number of inflammatory conditions. These conditions range from those associated with an intrinsic or primary defect in MCs due to inherited or acquired polymorphisms and mutations within the MC compartment; to diseases where MCs are activated and recruited through an extrinsic mechanism such as formation of antigen-dependent and IgE-mediated MC activation; to clinical conditions where MCs are implicated through the release of MC mediators but where the mechanism of activation and recruitment is not well understood, thus “idiopathic” (Table I)[108].

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There are several well characterized molecular aberrancies affecting the MC compartment. Some are associated with recognized clinical diseases due to effects on MC proliferation and survival, MC reactivity or MC mediator production. Mastocytosis is perhaps the most widely known disease associated with a primary defect in the MC compartment. It is a clonal disease involving expansion of tissue MCs. Mastocytosis is most commonly associated with an acquired gain-of-function *KIT* p.D816V missense variant, resulting in ligand-independent MC activation. This leads to both unrestrained growth of MCs and a lowered threshold for activation. Individuals with mastocytosis may present with flushing, pruritus, gastrointestinal complaints or systemic anaphylaxis that may occur following exposure to Hymenoptera venom or for unidentified reasons. Mastocytosis may present as cutaneous disease only, or as a systemic disease with or without cutaneous manifestations[109].

A group of patients with recurrent anaphylaxis has clonal MCs as demonstrated by evidence of one or two minor criteria for mastocytosis including aberrant MC morphology, CD25 expression and/or presence of the *KIT* D816V point mutation. By consensus, such patients are currently said to have monoclonal MC activation syndrome (MMAS)[110].If the marrow findings are observed in the absence of evidence of systemic MC activation, the term monoclonal MC disorder of uncertain significance has been suggested.

There are now a growing number of heritable genetic conditions that lead to increased MC reactivity. One such example is manifested as a physical urticaria. A missense substitution from cysteine to tyrosine (pC492Y) in the adhesion G protein-coupled receptor E2 (ADGRE2) is present in autosomal dominant vibratory urticaria (VU) characterized by localized hives and systemic manifestations in response to a local stimulus of frictional nature[111]. ADGRE2 (CD312) belongs to a large family of adhesion GPCRs generally with an extracellular domain facilitating interactions with proteins from the extracellular matrix. In this case, activation of MCs after a vibratory stimulus is evidenced by MC degranulation and increase in histamine in the venous blood from the affected areas. Thus, ADGRE2 functions as a mechanoreceptor and induces cutaneous MC degranulation. Although the physiological relevance of the limited MC responses to friction in normal individuals is not completely understood, possibilities are that ADGRE2 may alert both resident and immune cells to combat potential injury and wound healing. It may also play a role in pain modulation and perhaps help sense a parasite migrating through dermal tissues.

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Hereditary alpha tryptasemia (HαT) is a term used to describe a genetic trait caused by an increased TPSAB1 copy number encoding alpha-tryptase that leads to elevated basal serum tryptase levels in 4-6 % of Western populations[112,113]. Individuals with multiple duplications of alpha tryptase are reported tohave a higher risk for severe anaphylaxis. Further, the prevalence of HαTin clonal MC disease is twice that of the general population and may be a biomarker for severe mediator-related symptoms in those with mastocytosis[114,115]. Recent mechanistic studies have demonstrated that unique enzymatic properties of alpha-tryptase containing heterotetrameric tryptases may contribute to this association[116].At present, HαT is thus best thought of as a disease-modifying genetic finding.

Extrinsic or secondary MC activation occurs primarily in allergic diseases, diseases associated with complement activation, and in association with activation of MCs through MRGPRX2. Symptoms may be infrequent to frequent and resultant disease sporadic or chronic depending on the activating mechanism. The immediate effects of MC degranulation, if localized to skin, include a wheal and flare reaction or, in airways, contraction of airway smooth muscle, mucus secretion, and an increase in vascular permeability. If systemic, the results may include severe hypotension and extensive vascular leakage. The early responses often transition into a late phase reaction hours later associated with an influx of circulating cell types which promote further inflammation.

The idiopathic MC category includes urticaria, angioedema and anaphylaxis where there is no identifiable etiology, but where MC activation is documented through MC mediator release or evidence of MC degranulation in tissues involved (Table I). The term idiopathic MC activation syndrome (Idiopathic MCAS) has been applied as a diagnosis for individuals who present with such episodic allergic-like signs and symptoms such as flushing, urticaria, diarrhea, and wheezing involving two or more organ systems, where the etiology is unknown[117]. Diagnostic criteria include response to anti-mediator therapy and an elevation in a validated urinary or serum marker of MC activation, such as serum tryptase with an episode. Primary and secondary MC disorders must be eliminated as possible causes of the clinical findings. However, the search should continue for the etiology of these idiopathic disorders including the possibility that MC activation may relate to a yet-to-be identified endogenous or environmental stimulus or an intrinsic MC defect resulting in a hyperactive MC phenotype.

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The development of mast cell-targeted treatments

Mast cell mediators as targets of treatment

MC-targeted treatments are primarily developed for chronic urticaria (CU), mastocytosis, and allergies, because of the critical role that MCs play in their pathogenesis[118,119]. The evolution of MC-targeted treatments started, more than 70 years ago, with the development of drugs that inhibit the effects of individual MC mediators, first histamine, followed by prostaglandins and leukotrienes[120]. Modern antihistamines that act on the histamine 1 receptor are superior to first generation ones in their binding affinity, specificity, and risk/benefit profiles. Antihistamines that act on the histamine 4 receptor have shown promising results, and several such compounds are currently being developed for the treatment of MC-driven diseases[121]. Recently a new approach to inhibit tryptase, the major MC protease, with an anti-tryptase antibody was tested in preclinical primate studies[122]. The therapeutic area is initially severe asthma where MCs are implicated[123].

However, to target one MC mediator or one of its receptors comes with an inherent limitation, i.e., it prevents only the effects of one MC mediator or receptor. Activated MCs release many different mediators (acting on even more receptors) that are held to contribute to the development of signs and symptoms in patients with urticaria, mastocytosis and other MC-mediated diseases.

Treatments that inhibit mast cell activation

More recent attempts to target MCs therapeutically aim at the inhibition of activating signals and receptors (Figure 6). These include, for example, the high affinity receptor for IgE, FcεRI, as well as the intracellular signals involved in translating receptor activation into degranulation and mediator release. Examples for the latter include Bruton's tyrosine kinase (BTK) and spleen associated tyrosine kinase (SYK) (Figure 7). Inhibitors of BTK or SYK inhibit the degranulation of human MCs induced via FcεRI. Two BTK inhibitors, Fenebrutinib and Remibrutinib, as well as the SYK inhibitor GSK2646264 are currently under development for the treatment of patients with CU (Figure 7).

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In chronic spontaneous urticaria (CSU), the activation of skin MCs via FcεRI, either by IgE to autoallergensor by autoantibodies to its alpha chain, is held to drive the development of signs and symptoms, itchy wheals and angioedema, in most patients[124-126]. Treatment with omalizumab, an anti-IgE antibody, is effective in CSU[127] and prevents MC activation by reducing the levels of free IgE and FcεRI expression. Newer anti-IgEs, including ligelizumab and GI-301, with higher affinity to IgE than omalizumab, are in clinical development[128].

Several other activating signals and receptors are held to contribute to the activation and degranulation of MCs and, thereby, to the development of signs and symptoms of MC-driven diseases. These include the receptors for thymic stromal lymphopoietin (TSLP), IL-33, IL-4, IL-13, IL-5, and complement C5a as well as MRGPRX2. For all of these receptors, inhibitory compounds (or compounds that inhibit their ligands) are currently in clinical development for MC-driven diseases, primarily CU[129].

The benefit of targeting MC-activating signals, receptors, and pathways is that this approach protects from the release and, therefore, the effects of all MC mediators rather than only one. The limitation is that only one of many pathways of MC activation and degranulation is shut down. Other pathways are untouched, remain viable, and can lead to MC activation, as they often do in most MC-driven diseases.

Treatments that silence mast cells

MCs express several inhibitory receptors[130]. Their ligand engagement inhibits MC activation and degranulation, thereby silencing MCs. Inhibitory MC receptors, including Siglec-8 and CD200Ra, and therapeutic antibodies that engage them are currently under development for CU (Figure 6). The Siglec-8-targeted antibody AK002 inhibits MC activation (and depletes eosinophils) and showed promising results in the treatment of omalizumab-naïve and omalizumab-refractory CSU, symptomatic dermographism, cholinergic urticaria, and systemic mastocytosis.Several endogenous ligands for Siglec-8 have been identified. Siglec-8 preferentially binds the sialoside glycans 6′-sulfo-sialyl-Lewis X and 6′-sulfo-sialyl-N-acetyl-D-lactosamine. However, it is currently unknown how relevant they are for the inhibition of mast cells in vivo[131,132]. The CD200Ra-targeted antibody LY3454738 is also under development for CU (Figure 6).

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Treatments that reduce mast cell numbers

The KIT receptor is the key driver of MC differentiation, migration, proliferation and survival. The inhibition of KIT or SCF leads to MC apoptosis and reduced MC numbers. Compounds that target KIT or SCF are currently explored for the clinical development in MC-driven diseases including CU and mastocytosis[133]. For example, the antibody CDX-0159, which specifically binds the extracellular dimerization domain of KIT, was shown to induce profound and sustained suppression of plasma tryptase, indicative of systemic MC ablation (Figure 6). CDX-0159 is currently under development for CU. Examples for the development of oral KIT-targeted treatments include Imatinib, Nilotinib, Midostaurin, and Avapritinib (Figure 7), all of which show efficacy in mastocytosis linked to reductions in MCs and/or serum tryptase levels. To note, Imatinib and Nilotinib inhibits wild type KIT, but do not affect D816V-mutated KIT, and thus can only be used for D816V negative cases of mastocytosis.In addition, there are a number of potential targets, apart from KIT, that in experimental models have been shown to regulate mast cell numbers and activity [109,133,134].

Animal models to study mast cell function

Numerous types of animal models, primarily in the mouse, have been utilized throughout the years to outline the contribution of MCs in diverse pathological settings. In the first generation of such models, MC-deficiency in the mouse and rat was due to various mutations in *Kit*, i.e. the receptor for SCF. Since SCF is an essential growth factor for MCs, defects in *Kit* result in an essentially complete absence of MCs. However, KIT is also expressed by a number of other cell types, and it has therefore been challenging to ascertain that consequences of *Kit* defects are indeed explained by an impact on the MC niche as opposed to off-target effects on other populations (reviewed in[135,136]). To account for these issues, new mouse models of MC-deficiency, independent of *Kit*, have been developed (Table 2). These include mice where MC deficiency is driven by Cre recombinase expression under the control of MC-specific promoters. In one strategy, Cre recombinase was driven by the promoter for *Mcpt5*, a gene specifically expressed by CTMCs. These mice can then be crossed with *R26*^{DTA} mice, leading to constitutive MC deficiency due to MC-specific expression of diphtheria toxin (DT)[137]. Alternatively, the mice can be crossed with

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the *iDTR* line, leading to MC-specific expression of the DT receptor; treatment of these mice with DT will thus lead to conditional depletion of MCs[137]. In another approach, MC deficiency was accomplished by inserting Cre under the control of the promoter for Cpa3, a gene expressed predominantly by MCs[138]. This leads to constitutive MC depletion, apparently due to Cre-mediated genotoxicity, but also to a substantial reduction of basophils, the latter in agreement with studies showing that basophils express low levels of Cpa3. The Cpa3 promoter was also exploited to generate a mouse strain in which the Cre-LoxP recombination system was used for deletion of the gene coding for the anti-apoptotic factor Mcl-1. This led to an essentially complete absence of MCs but also to a major reduction in basophils[139]. MC depletion has also been accomplished in a model where the DT receptor gene was expressed under the control of a MC-specific IL-4 enhancer element[140]. Another strategy was to insert the DT receptor and bright red td-Tomato fluorescent protein genes into the gene coding for the β chain of FcεRI, which is expressed by basophils and MCs. This approach can be used for depletion of MCs and basophils and also as an elegant tool to visualize MCs/basophils in vivo[141]. More recently, a mouse line with reduced numbers of MMCs (CTMCs were not affected) was generated by expressing Cre under the control of the baboon-α-chymase gene, and crossing these to mice with a floxed allele of Mcl-1[142].

By using these *Kit*-independent models of MC deficiency, important insight into the biological function of MCs has been obtained. As expected, the use of *Kit*-independent mouse models for MC-deficiency has firmly confirmed the essential role of MCs in allergic responses[138,143]. Moreover, recent studies have shown that MCs can have a role in melanoma dissemination[144], cutaneous lymphoma[145], collagen-induced arthritis[146], bone fracture-associated inflammation [147] and bone healing[148]. It was also demonstrated that MCs aggravate osteoarthritis[149] and can mediate the detrimental impact of smoke components on asthmatic features[150]. Further, it has been demonstrated that MCs have a beneficial role in controlling bacterial clearance and promoting wound healing after *Pseudomonas aeruginosa* infection[151], whereas a detrimental impact of MCs was seen in skin infection by *Sporothrixschenckii*[152].

However, it is important to note that the use of these novel mouse models for MC deficiency has challenged some previous findings where a contribution of MCs in various pathologies has been implied. For example, recent findings based on *Kit*-independent mouse models for MC deficiency have questioned the role of MCs in certain models for autoimmune diseases[138] and obesity[153], as

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well as the proposed adjuvant activities of MCs[146]. Hence, a more nuanced view of how MCs are involved in pathological settings is currently emerging.

In addition to the various mouse models described above, recent efforts have resulted in the generation of mice in which the MC niche is populated by human MCs. This was accomplished by transplanting human hematopoietic stem cells into NOD-scid IL2R-$\gamma^{-/-}$ mice, and then promoting MC growth by administrating plasmids expressing human SCF, GM-CSF and IL-3. In these mice (denoted "humice" orNSG-SGM3), mature human MCs (co-expressing KIT and FcεRI) were detected in multiple tissues. These "humice" have so far mainly been used to study the role of MCs in anaphylaxis and cutaneous drug reactions[154-156], but also in the evaluation of new therapies, e.g., using a BTK inhibitor[157]. Moreover, functional human MCs could be developed from the bone marrow of these mice. Clearly, this humanized model has the potential to be used as a highly valuable tool to study human MC function. Another approach for studying the function of MCs is to evaluate mice deficient in various MC mediators, including the MC-restricted proteases, serglycin and histamine (reviewed in [16,123,158]).

Conclusion

Mast cell biology and their function in health and disease has been a faschinating subject for many researchers over the years. Many seminal findings have been made since the discovery of mast cells in the late 19th century (some of them given in Box 1), shedding light on the function of this ingenious cell. Nevertheless, there are many fundamental questions that still are awating an answer (some listed in Box 2). With the rapid developments in methodology, systems biology etc., in combination with more relevant animal models, experimental human studies and clincal investigations, we can foresee a rapid development in mast cell research the coming years that will not only give insights about their biology but also a better understanding on their role in different diseases.

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Table 1. Classification of mast cell mediated diseases*

1. Primary (Intrinsic)
   a. Mastocytosis
   b. Monoclonal mast cell activation syndrome (MMAS)
   c. Autosomal dominant vibratory urticaria (VU)
2. Secondary (Extrinsic)
   a. Allergic disorders (FcεRI mediated)
   b. Mast cell activation through the high affinity IgG receptor (CD64)
   c. MRGPRX2 mediated reactions
   d. Diseases associated with complement activation (C3a, C5a)
3. Idiopathic#
   a. Anaphylaxis
   b. Angioedema
   c. Urticaria
   d. Idiopathic mast cell activation syndrome (Idiopathic MCAS)

*See text for explanation. Table is conceptually based on Table 1 in reference [108] which classifies mast cell activation into primary or secondary. Here, focus is on human mast cell-associated diseases, examples only

When no inciting stimulus is identified

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Table 2. KIT-independent constitutive and inducible mouse models of MC deficiency.

Model, designation	**Principle**	**Type of MC affected**	**Basophils affected**	**Ref**
Constitutive				
Mcpt5-Cre; R-DTA	Cre expression under the control of the Mcpt5 promoter; Cre-driven expression of DT	CTMCs	No	[137]
$Cpa3^{Cre+}$ ("Cre-Master")	Cre expression under the control of the Cpa3 promoter	CTMCs + MMCs	Yes (~60% reduction)	[138]
Cpa3-Cre; $Mcl\text{-}1^{fl/fl}$ ("Hello Kitty")	Mcl-1 deletion under the control of the Cpa3 promoter	CTMCs + MMCs	Yes (~60-80% reduction)	[139]
Chm-Cre; $Mcl\text{-}1^{fl/fl}$	Mcl-1 deletion under the control of the baboon-α-chymase gene	MMCs reduced; CTMCs normal	Not determined	[142]
Inducible				
Mas-TRECK	DT receptor expressed under the control of a MC-specific IL-4 enhancer element	CTMCs; MMCs not determined	Transient depletion; recovery of basophil populations 12 days after DT	[140]

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			administration	
Mcpt5-Cre; iDTR	DT receptor expressed under the control of the Mcpt5 promoter	CTMCs	No	[137]
Red mast cell and basophil (RMB) mouse	DT receptor and bright red td-Tomato fluorescent protein expressed under the control the β chain of FcεRI (Ms4a2)	CTMCs; MMCs not determined	Transient depletion; recovery of basophil populations 12 days after DT administration	[141]

ND, not determined; DT, diphtheria toxin; CTMC, connective tissue-type MC; MMC, mucosal-type MC

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Box 1 Major milestone discoveries in the field of mast cell biology

1863 Friedrich von Recklinghausenidentify granulated cells in unstained connective tissues from different species

1878 Identification and description of mast cells by Paul Ehrlich[159]

1937 Heparin is present in mast cells[160,161]

1949 Description of systemic mastocytosis[162]

1953 Mast cells contain histamine[163]

1956 Demonstration of the involvement of mast cells in anaphylaxis[164]

1966 Description of mast cell heterogeneity, i.e, mucosal mast cells and connective tissue mast cells[68,69]

1966 & 1967 Identification and characterization of IgE[165-167]

1970 & 1972 Slow reacting substance of anaphylaxis (SRS-A), later shown to be leukotrienes, was demonstrated to be released after IgE-receptor activation[168,169]

1977 Mast cells develop from bone marrow cells[46]

1981 Identification of tryptase as the predominant mast cell protease[170,171]

1982 Prostaglandin D_2 shown to be released from mast cells[172]

1987 Mast cells can express and secrete cytokines[173,174]

1989 Characterization of the high-affinity IgE-receptor[175,176]

1989 The central nervous system affects mast cell reactivity[177]

1990 Identification, cloning and characterization of SCF[178]

1992 Demonstration that SCF is the main growth and maturation factor for human mast cells[67,179-181]

1995 Identification of the D816V *KIT* mutation in systemic mastocytosis[182]

2004 & 2006 Mast cell proteases detoxify endogenous peptides and venoms[183,184]

2015 Identification of MRGPRX2 as a receptor causing pseudoallergic reactions[3]

2018 Insight into the prenatal development of mast cells[43,44]

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Box 2 Future research perspectives

• To determine if MCs from different hematopoietic waves exhibit distinct functions.

• To determine the developmental relationship between the MC trajectory and other myeloerythroid lineages. Is it consistent in health and disease?

• To identify mechanisms behind an increase in MCs at a site of inflammation: local proliferation, survival and/or progenitor recruitment to the site of inflammation? What chemotactic factors drive MC progenitor recruitment in vivo?

• To get a better understanding of MC heterogeneity in different tissues and to define the mechanisms that regulate MC plasticity.

• To understand the mechanisms for differential release of MC mediators.

• To decipher the contribution of MCs and their mediators in diseases beyond primary MC diseases and allergy.

• To understand mast cell activation syndrome (MCAS). What are the mechanisms? How can it be diagnosed and treated?

• Development of drugs that selectively target MCs.

• Development of new KIT-independent MC deficient models (mouse, but also other species such as rat, ferret and guinea pig that might be better for specific diseases), in which MC functions and contributions to health and disease can be specifically investigated.

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Figure legends

Figure 1. Mast cell activation pathways and release of mediators. Mast cells are endowed with a palette of receptors that can recognize endogenous and exogenous products and release different sets of mediators dependent on the receptor involved. Upon recognition mast cells react by releasing mediators through one or several pathways: 1) degranulation and release of granules containing e.g., histamine, heparin, proteases and cytokines; 2) release of newly synthesized lipid mediators such as PGD_2, $LTC_4/D_4/E_4$, TXA_2 and 15-HETE; 3) release of de novo synthesized cytokines, chemokines, growth factors and interferons; 4) release of extracellular vesicles, e.g., exosomes, containing proteins, mRNA and miRNA.

Figure 2. Differential release of mediators. Depending on ligand – receptor interaction mast cells react by releasing different sets of mediators, those stored in granules, newly synthesized and secreted lipid mediators, and *de novo* produced proteins. Mast cells activated with allergen binding to IgEbound to the high-affinity IgE receptor, FcεRI, induces degranulation, secretion of lipid mediators and different types of proteins, including cytokines and chemokines In contrast, the alarmin IL-33 does not induce degranulation, but causes secretion of lipid mediators, cytokines and chemokines [185]. The costimulatory molecule CD30 induces secretion of only cytokines and chemokines [23].Viral infection leads to a variety of responses, depending on the type of virus encountered [15]. For example, respiratory syncytial virus infection induces production of type I interferons and chemokines [24].

Figure 3. Putative symptoms induced by mast cell derived mediators. Activation of mast cells causes the release of a multitude of mediators that can act on cells or substrates in different organs and systems which might create clinically relevant symptoms as depicted in the figure.

Figure 4. Models representing mast cell differentiation in hematopoiesis. (A) The tree-like model represents hematopoiesis as a strict series of branching events, in which cell lineage potential is lost at defined checkpoints along differentiation from hematopoietic stem cells to lineage-committed cells. The position of the mast cell branch in this model is controversial. (B) The hematopoietic landscape model represents a continuum of single differentiating progenitors, in which there are multiple paths from hematopoietic stem cells to each lineage's entry point. Ba,

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basophil; Eo, eosinophil; Ery, erythrocyte, Gr, granulocyte(which commonly refers to basophils, eosinophils, and neutrophils), HSC, hematopoietic stem cell; Ly, lymphocyte; MC, mast cell; MK, megakaryocyte; Mo, monocyte, Neu, neutrophil.

Figure 5. Fate decisions in the life of the mast cell. Mast cell progenitors (MCp) in the bone marrow (1) are entering the blood stream and are recruited into the tissue (2). During this process the cells have to avoid premature degranulation (2). In the tissue (3) MCs can degranulate in three possible modes, either by full exocytosis, compound exocytosis or by kiss-and-run exocytosis.

Figure 6. MC-targeted treatments under development. Examples of treatments currently under development that target mast cell mediators, receptors or their signaling molecules.

Figure 7.Intracellular targets to inhibit MC-activating receptors FcεRI and KIT. Small molecular inhibitors that targetsSyk or BTK down-stream of FcεRI inhibits the down-stream signaling. The KIT-inhibitors target the kinase activity of the receptor.Imatinib and Nilotinib targets only wild type KIT, while Midostaurin and Avapritinib also targets D816V-mutated KIT that is commonly found in systemic mastocytosis.

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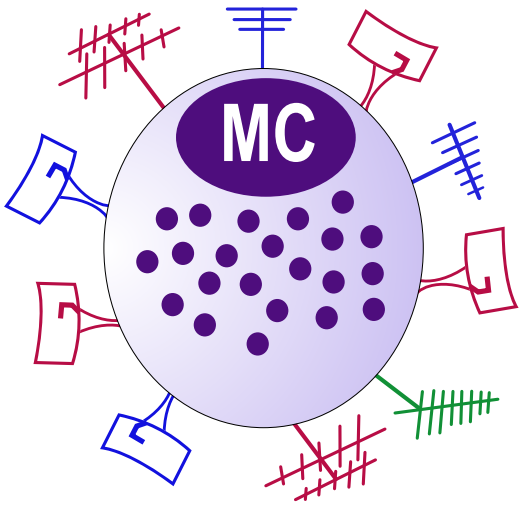
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1. Degranulation
(granules 0.2 - 0.8 µm)

Mediators, e.g.

- Histamine
- Heparin
- Tryptase
- Cytokines



4. Extracellular vesicles,
e.g. exosomes (30 - 150 nm)

- Proteins
- mRNA
- miRNA

3. De novo protein synthesis, e.g.

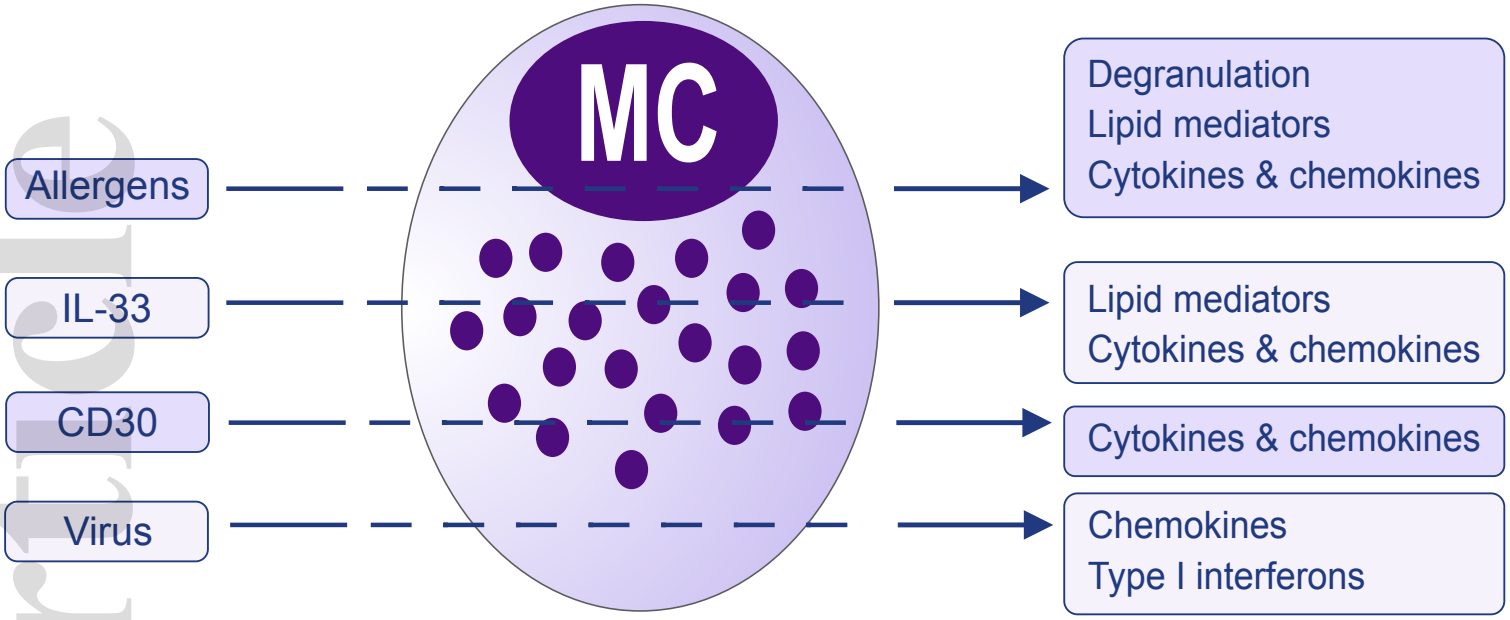
- Cytokines (IL-4, IL-13, GM-CSF)
- Chemokines (CXCL8, CCL2)
- Growth factors (VEGF)
- Interferons (IFN-β, IFN-γ)

2. De novo lipid biosynthesis, e.g.

- PGD_2
- $LTC_4/D_4/E_4$
- TXA_2
- 15-HETE

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Figure 1_Nilsson et al.

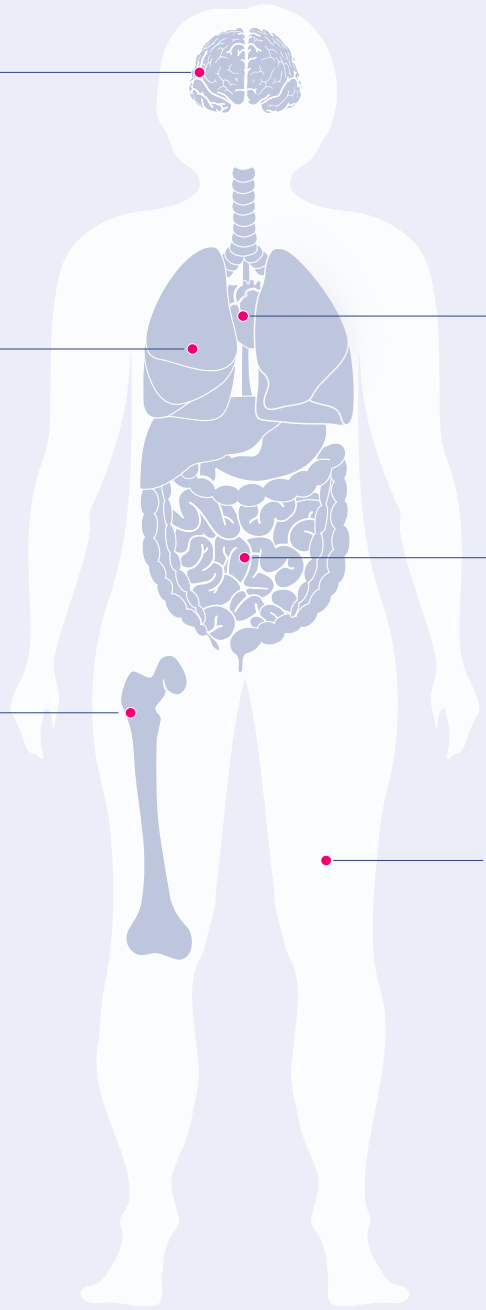


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Figure 2_Nilsson et

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Neurologic
- Anxiety
- Decreased concentration
- Depression
- Migraine

Respiratory
- Nasal congestion
- Nasal pruritus
- Bronchospasm
- Swelling
- Wheezing

Musculoskeletal
- Aches
- Bone pain
- Osteopenia
- Osteoporosis

Cardiovascular
- Hypotension
- Syncope
- Tachycardia

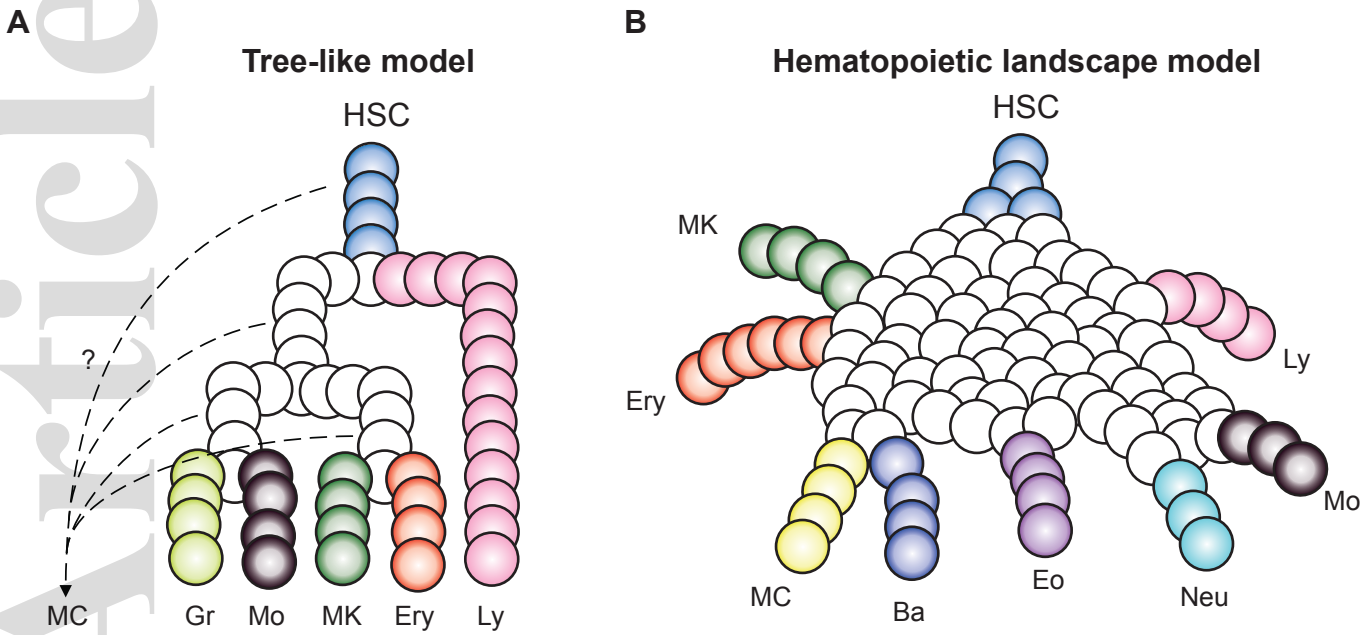
Digestive
- Abdominal pain
- Diarrhea
- Esophageal reflux
- Nausea and vomiting

Cutaneous
- Flushing
- Pruritus
- Urticaria
- Angioedema

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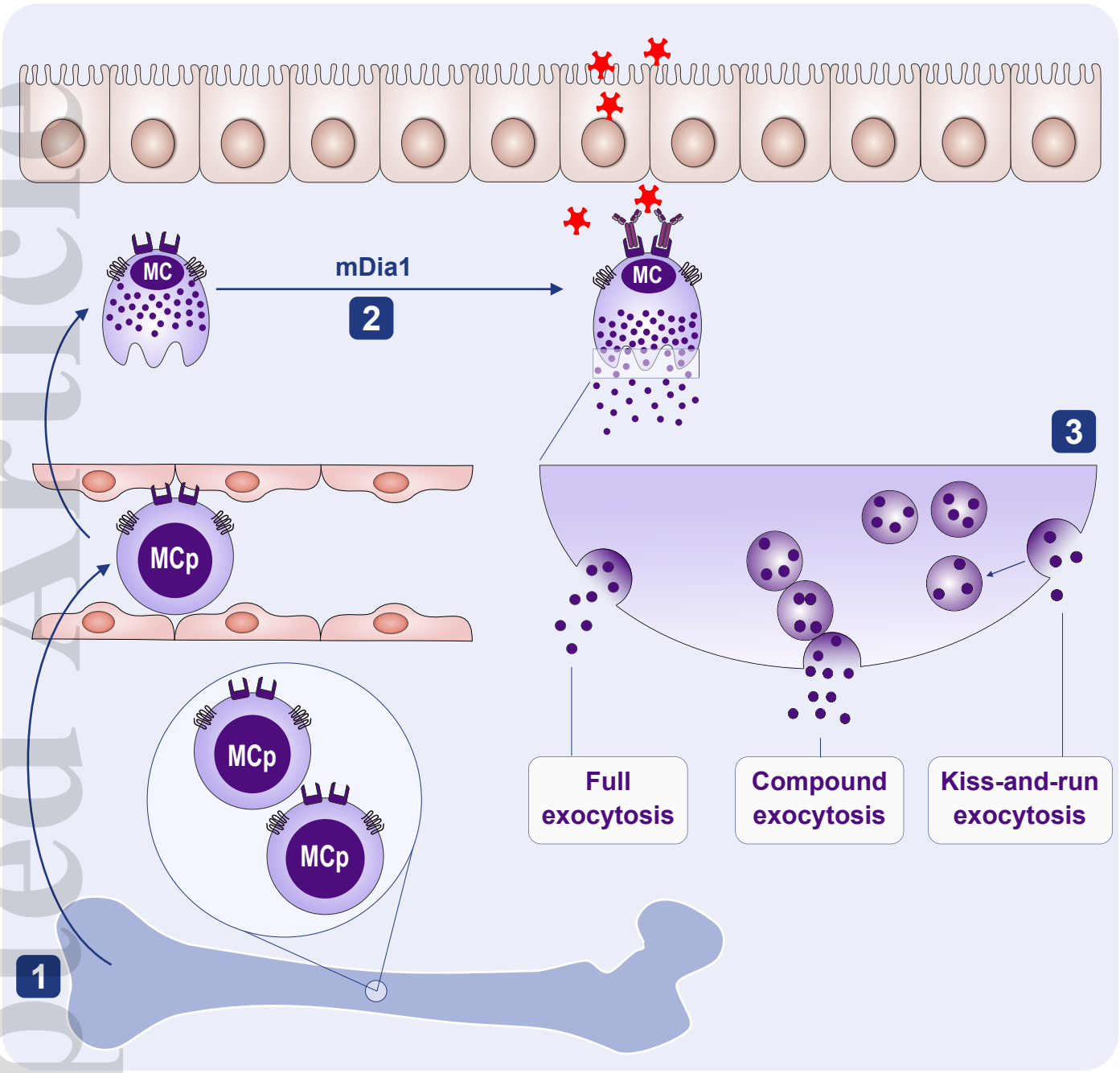
Figure 3_Nilsson et

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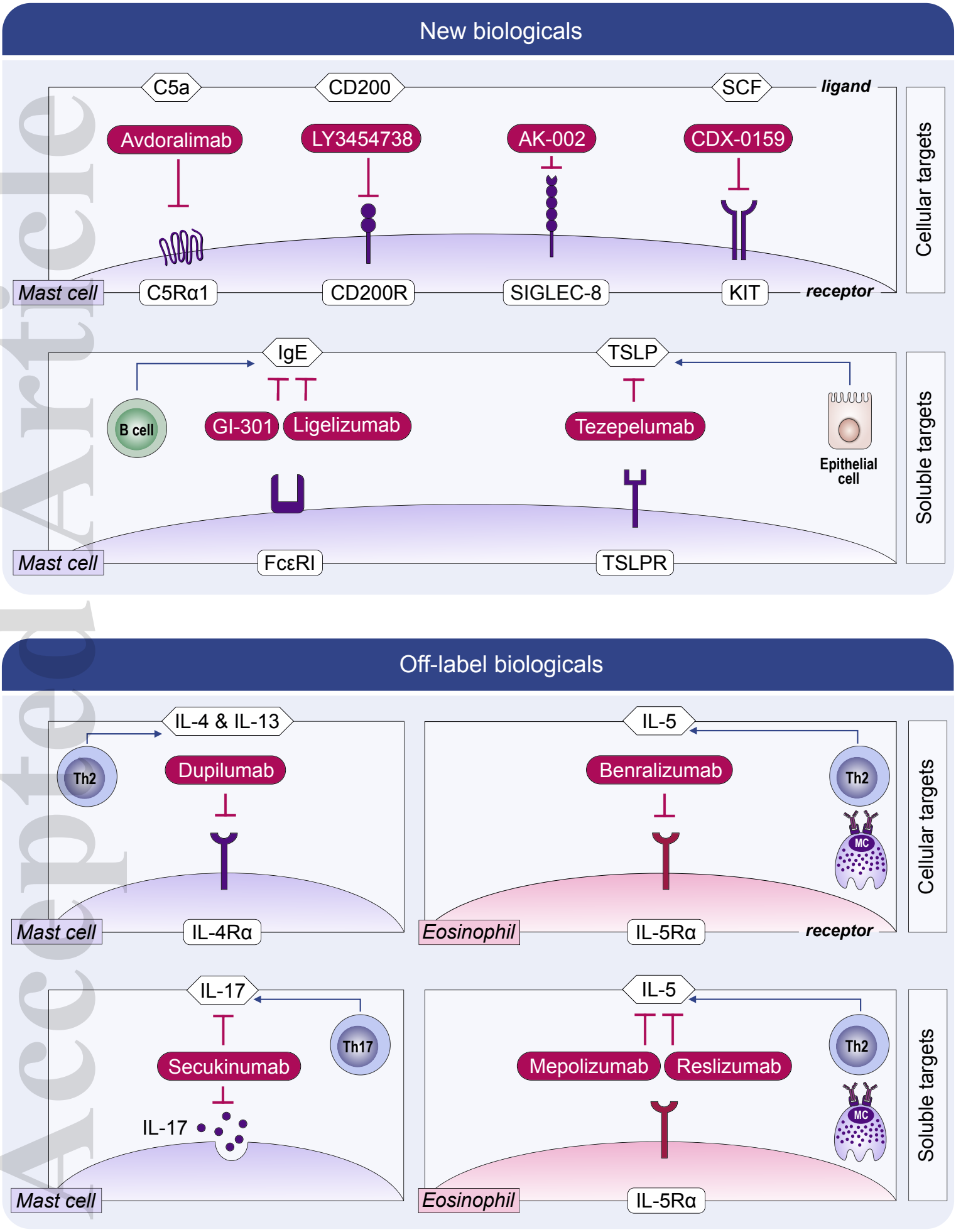
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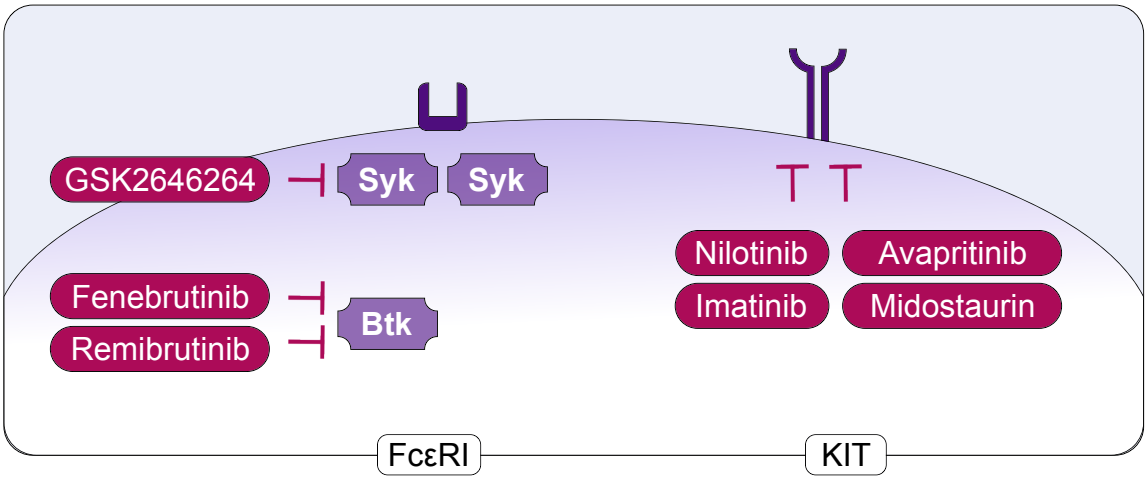
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Figure 7_Nilsson et