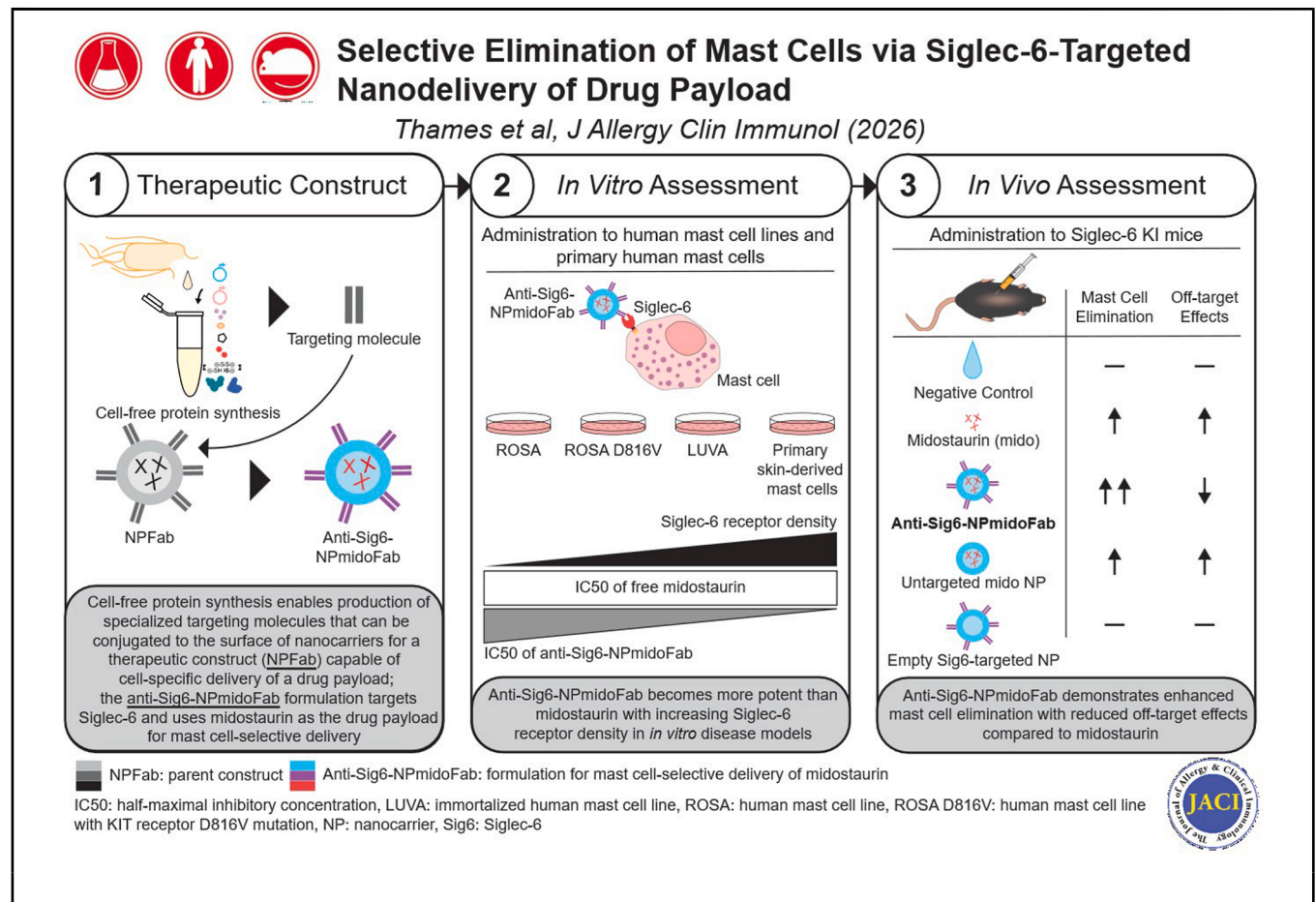


Selective elimination of mast cells via Siglec-6-targeted nanodelivery of drug payload

Ariel Helms Thames, MD, PhD, Clayton H. Rische, PhD, Rebecca A. Krier-Burris, MS, Yun Cao, MS, Andrew C. Hunt, PhD, Natalie R. Klug, et al

GRAPHICAL ABSTRACT



Capsule summary: Anti-Sig6-NPmidoFab is a targeted nanotherapy, enabled by cell-free protein synthesis, that achieves selective mast cell elimination, offering a new treatment strategy for mast cell-mediated disease with potential for broad application.

Selective elimination of mast cells via Siglec-6-targeted nanodelivery of drug payload

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Background: Mast cell elimination through sialic acid immunoglobulin-like lectin 6 (Siglec-6) is a promising, yet minimally explored strategy for diseases characterized by mast cell overactivity or overproduction.

Objective: We combined nanotechnology with cell-free protein synthesis to develop a therapeutic strategy (anti-Siglec-6-NPmidoFab) for selectively eliminating mast cells.

Methods: Nanocarriers are loaded with the protein kinase inhibitor midostaurin and conjugated with cell-free protein synthesis-enabled clickable, synthetically dimerized anti-Siglec-6 antibody fragments to selectively target Siglec-6-expressing mast cells.

Results: Using human mast cell lines and primary human mast cells as well as a Siglec-6 knock-in mouse model and a humanized mast cell malignancy mouse model, we demonstrated selective elimination of Siglec-6-positive mast cells with reduced off-target effects on Siglec-6-negative cells.

Conclusion: This approach offers new treatment strategies for malignant and nonmalignant mast cell-mediated disease with potential for broad application. (*J Allergy Clin Immunol* 2026;■■■:■■■-■■■.)

Key words: Nanotherapy, Siglec-6, cell-free protein synthesis, mast cells, cancer, mastocytosis

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The prevalence of mast cell malignancies such as mastocytosis is estimated to be 1 or 2 per 10,000, although they are likely underdiagnosed because of their rarity and nonspecific symptoms.¹ Mastocytosis typically presents with urticaria pigmentosa, flushing, diarrhea, and/or tachycardia. It can be classified as either cutaneous mastocytosis or systemic mastocytosis, with prognosis ranging from indolent to rapidly deteriorating, the latter being associated with high rates of mortality.²⁻⁴ This rare disease can be devastating. The estimated disability-adjusted life year per 100,000 inhabitants of indolent systemic mastocytosis is 89 to 168, on par with that of leukemia, lymphoma, and psoriasis.⁵

Mast cell malignancies are driven by aberrant mast cell behavior and excessive proliferation. Over 90% of systemic mastocytosis cases are associated with a gain-of-function D816V mutation in exon 17 of the cytoplasmic domain of the KIT receptor (CD117), resulting in constitutive signaling. KIT is a tyrosine kinase receptor activated by stem cell factor, which leads to mast cell proliferation and survival.^{6,7}

The current standard of treatment relies on tyrosine kinase inhibitors (TKIs), most of which have some degree of off-target effects. Midostaurin (Rydapt; Novartis Pharmaceuticals) is a small-molecule multiple TKI that in 2017 was the first drug of its kind approved for treating systemic mastocytosis.^{2,8,9} Although effective, midostaurin has broad kinase inhibitory activity, leading to several adverse effects, such as lymphocytopenia.^{2,10,11} It also has low aqueous solubility and poor bioavailability and must be dosed orally.¹² In more recent years, other small-molecule drugs have been investigated on the basis of the binding and targeting strategy of midostaurin. Imatinib is the first selective TKI to be approved but depends on the wild-type form of KIT for binding.¹³ Avapritinib (Aykavit; Blueprint Medicines) and bezuclastinib are more recent TKI variations designed to have more selective activity against the mutant D816V form of KIT.^{8,14,15}

New treatments that are mutation agnostic with minimal off-target effects and favorable bioavailability are needed to achieve mast cell inhibition or elimination. Here, we introduce a platform for making targeting molecules that enables a cell-selective therapeutic construct. The platform relies on cell-free protein synthesis (CFPS), which involves isolating transcription and translation machinery from *Escherichia coli* in the form of a crude extract to express desired proteins.¹⁶⁻²⁶ CFPS allows *de novo* production of antibody fragments (Fabs) capable of synthetic dimerization (sd) and click chemistry conjugation (azido-modified, synthetically dimerized fragment antigen-binding antibodies [azido-sdFabs]).²⁷⁻³⁰ These targeting molecules can then be conjugated to the surface of poly(ethylene glycol)-block-poly(propylene sulfide) (PEG-b-PPS) nanoparticles (NPs) shown

Abbreviations used

Azido-sdFab:	Azido-modified, synthetically dimerized fragment antigen-binding antibody
CD45:	Leukocyte common antigen
CFPS:	Cell-free protein synthesis
Cpa3:	Carboxypeptidase A3
DBCO:	Dibenzocyclooctyne
DSPE:	1,2-Distearoyl-sn-glycero-3-phosphoethanolamine
Fab:	Antibody fragment
KI:	Knock in
LUVa:	Immortalized human mast cell line from Laboratory of University of Virginia
NP:	Nanoparticle
PEG:	Polyethylene glycol
PEG-b-PPS:	PEG-block-poly(propylene sulfide)
ROSA D816V:	ROSA cells with mutated KIT
ROSA WT:	ROSA cells with wild-type KIT
sd:	Synthetically dimerized
Sig6-targeted NPs:	Empty Siglec-6-targeted micelles without drug payload
Siglec:	Sialic acid immunoglobulin-like lectin
TKI:	Tyrosine kinase inhibitor

to improve bioavailability and the nanocarrier can be loaded with a drug payload, such as midostaurin, for cell-selective delivery.³¹⁻³⁵

We next considered a cell type for proof-of-concept testing of this construct. We chose malignant mast cells for this purpose, examining mast cell surface markers on which to base azido-sdFab specificity for nanocarrier targeting. Anti-KIT antibodies have been shown to have antiproliferative effects on normal mast cells but depend on a normally functioning KIT receptor, so they likely will not alter malignant mast cell survival and proliferation.³⁶ KIT is also expressed on several other cell types, including sperm cell progenitors, hair follicle pigment cells, hematopoietic stem cells, megakaryocytes, and primed CD8⁺ T cells. Adverse effects reported from KIT targeting include neutropenia, decreased sperm counts, and skin and hair depigmentation.³⁷⁻⁴⁵ As a promising alternative, we looked to a family of inhibitory immunoreceptors known as sialic acid-binding immunoglobulin-like lectins (Siglecs) to identify a mast cell selective target. Siglec-3, -6, -7, -8, and -9 are expressed on human mast cells.⁴⁶⁻⁵⁰ Mast cells share Siglec-3 (CD33) expression with several myeloid-derived immune cell types, Siglec-8 expression with eosinophils, and Siglec-9 with several myeloid- and lymphoid-derived immune cells.⁵¹ Antibody-drug conjugates targeting Siglec-3 and Siglec-8 have shown promise in promoting malignant mast cell death.⁵²⁻⁵⁵ Mast cells share Siglec-6 expression with placental trophoblasts, which are only present during pregnancy.^{46,56} Recent data have also found evidence of Siglec-6 expression with some hematologic malignancies and possibly memory B cells.⁵⁷ Anti-Siglec-6 antibodies have shown promise in promoting mast cell inhibition and depletion through antibody-dependent cellular phagocytosis and were well tolerated in a phase 1 trial.^{58,59} We selected Siglec-6 as a target and modeled our azido-sdFabs after an anti-Siglec-6 antibody known to be rapidly internalized for more efficient payload delivery.⁵⁸

Overall, we sought to develop a therapeutic construct, NPFab, for cell-selective delivery of a drug payload. We formulated the

construct for the treatment of mast cell malignancies (anti-Sig6-NPmidoFab) for proof-of-concept testing. In our anti-Sig6-NPmidoFab formulation, midostaurin is loaded into PEG-b-PPS nanocarriers, which are then conjugated with CFPS-enabled anti-Siglec-6 azido-sdFab fragments for a mutation agnostic, mast cell-targeted, cytotoxic nanotherapy. In several relevant disease models, including a Siglec-6 knock-in (KI) mouse model, we show enhanced and selective elimination of mast cells by anti-Sig6-NPmidoFabs loaded with midostaurin compared to midostaurin alone, thus offering a safer, cell-targeted therapeutic approach potentially involving a range of drug payload delivery strategies for the treatment of mast cell malignancies and other mast cell-mediated diseases.

METHODS

Deidentified human skin samples were obtained under an institutional review board waiver (STU00218089). All protocols involving murine models were approved by Northwestern University's institutional animal care and use committee. Detailed methods are provided in the Methods section in this article's Online Repository available at www.jacionline.org.

RESULTS**Design and assembly of Siglec-6-targeting molecules**

By using CFPS, we overcome challenges of existing approaches for nanocarrier targeting, which rely on full-length antibodies or digested fragments of the antigen-binding region (Fabs). The large size of full-length antibodies poses binding-site limitations because moderate and high densities can compromise NP integrity as a result of steric stress and destabilization by the larger protein. Digestion of Fab fragments are often incomplete, rely on denaturing conditions, and leave limited chemistries for NP conjugation. The use of CFPS enables production of Fab fragments designed to include a leucine zipper motif for reliable assembly of Fab fragments through synthetic dimerization (sd) and to permit incorporation of a noncanonical amino acid for click chemistry conjugation.^{60,61} We also express Fabs under oxidizing conditions to further support assembly by disulfide bond formation. By using a recoded *E coli* strain where one of the stop codons (TAG) has been freed up by genome engineering,^{16,30} we can include a TAG codon for incorporation of azido-modified phenylalanine, which provides the azide click handle for conjugation between the targeting molecule and NP. Click chemistry is a conjugation reaction that occurs between an azide and an alkyne, forming a covalent bond, and is known for its efficiency and bio-orthogonality.²⁷⁻²⁹ The resulting molecule is a clickable, synthetically dimerized Fab (azido-sdFab).

Anti-Siglec-6 azido-sdFabs are designed on the basis of the sequence of a commercially available anti-Siglec-6 antibody that is rapidly internalized.⁵⁸ They are encoded as separate heavy-chain and light-chain constructs on two separate plasmids. The constructs include (1) downstream complementary leucine zipper heterodimer domains (LZA and LZB) for synthetic dimerization, (2) affinity tags on the heavy chain for purification and analysis, and (3) a TAG codon for noncanonical azido-modified phenylalanine incorporation on the light chain to enable click chemistry conjugation. The opposing position of the affinity tag and TAG codon ensures that only assembled Fabs will be present on the

NP surface after purification and conjugation (Fig 1, A, and see Table E1 in the Online Repository available at www.jacionline.org). Two designs were made. The mouse azido-sdFab encodes the mouse kappa constant region for the light chain and mouse IgG₂a constant region for the heavy chain. The chimeric azido-sdFab encodes human kappa constant region for the light chain and the human IgG₁ constant region for the heavy chain. Constructs were expressed by CFPS (Fig 1, B) and visualized on a gel after incorporation of fluorescent lysine (FluoroTect) and before purification. The predominant band indicative of fully assembled product is visible at ~70 kDa along with smaller bands representing unassembled heavy and light chains. Some unassembled components are to be expected, but the predominant product is the fully assembled Fab fragment (Fig 1, C). Residual fluorescent dye in the figures can be seen as smeared lanes, as is typical with the FluoroTect technology.

In vitro binding of anti-Siglec-6 azido-sdFabs

To confirm expression and assembly leads to functional azido-sdFabs capable of binding Siglec-6 *in vitro*, a bead-based AlphaLISA assay was used to detect binding. AlphaLISA is an in-solution ELISA that allows for high-throughput analysis of protein interactions.⁶¹ A cotitration was performed where commercially produced Siglec-6 containing an Fc tag associates with protein A donor beads and anti-Siglec-6 azido-sdFab expressed by CFPS associate with anti-flag acceptor beads. Successful binding is determined by the emission of light resulting from an energy transfer between donor and acceptor beads when brought in close proximity through interactions of their associated binding partners. Typically, signal is highest at some optimal concentration and decreases as concentrations move away from the central optimum. At higher concentrations, this is due to the Hook effect; at lower concentrations, this is due to insufficient material. The pattern observed on the cotitration heat maps indicates successful binding of both mouse and chimeric azido-sdFab to Siglec-6 (Fig 1, D).

Cross-reactivity of anti-Siglec-6 azido-sdFabs with other selected Siglecs

To verify that anti-Siglec-6 azido-sdFabs are specific to Siglec-6 and do not have cross-reactivity with other Siglecs expressed by mast cells, a similar AlphaLISA cotitration, narrowed to the optimal concentration for visualizing binding interactions, was performed with commercially produced Siglec-6, Siglec-8, and Siglec-3 (CD33). Siglec-8 and Siglec-3 were chosen as representatives of narrowly and broadly expressed Siglecs, respectively, that also share expression with mast cells. Successful binding was observed for azido-sdFabs against Siglec-6 (Fig 2), but not for Siglec-8 or Siglec-3, as indicated by signal levels near background when at least one binding partner was absent. This indicates that azido-sdFabs recognize Siglec-6 without off-target cross-reactivity with other mast cell-specific Siglecs, consistent with the binding patterns of the original mouse anti-human Siglec-6 monoclonal antibody from which the azido-sdFabs were based.

Preparation of anti-Sig6-NPmidoFabs

We prepared PEG-b-PPS micelle nanocarriers, which are small (typically 10-50 nm), monolayered nanocarriers loaded

with or without midostaurin. 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-polyethylene glycol-dibenzocyclooctyne (DSPE-PEG-DBCO) is an amphiphilic polymer that contains a strained alkyne and was incorporated into PEG-b-PPS micelles to prepare them for click chemistry conjugation to azido-sdFabs. DSPE-PEG2K-DBCO was successfully incorporated into the assembled nanocarrier structure without compromising particle size and homogeneity. Size measurements were consistently in a range of 18 to 25 nm with relatively low polydispersity, regardless of the weight percentage of DBCO-labeled molecules incorporated (0.5, 2, and 4 wt%; data not shown). Loading of midostaurin was verified by UV-Vis spectroscopy. Drug encapsulation was consistent across DSPE-PEG-DBCO compositions (see Table E2 in the Online Repository available at www.jacionline.org).

PEG-b-PPS micelles were then incubated with anti-Siglec-6 azido-sdFabs for click chemistry conjugation. To determine conjugation efficiency, micelles were incubated with fluorescently labeled azide to quantify the amount of unreacted DBCO. The conjugation efficiency, or percentage of available sites conjugated to the azido-sdFabs, is approximately 94% for PEG-b-PPS micelles composed of 0.5 wt% DSPE-PEG2K-DBCO and 81% for micelles composed of 2 wt% DSPE-PEG2K-DBCO (data not shown). The 0.5 wt% particles were used for further experiments. Purification and removal of unreacted azido-sdFabs was verified by free fluorescently labeled DBCO.

Anti-Sig6-NPmidoFabs reduce the viability of human mast cells and cell lines

To evaluate mast cell elimination *in vitro*, we utilized a human mast cell line capable of IgE-mediated degranulation (ROSA WT), a transfected version of this cell line bearing the most common mutation associated with mastocytosis (ROSA D816V),⁶² an immortalized human mast cell line (LUVa),⁶³ and normal primary human mast cells from skin.^{64,65} We performed cell viability MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assays after incubating human mast cell lines and primary human mast cells overnight with PEG-b-PPS micelles conjugated to anti-Siglec-6 azido-sdFabs that were or were not loaded with midostaurin. Anti-Sig6-NPmidoFabs loaded with midostaurin showed comparable mast cell killing to that of free midostaurin, while Siglec-6-targeted micelles without drug payload (empty Sig6-targeted NPs) had little to no effect. ROSA WT cell lines and ROSA D816V cells displayed approximately the same receptiveness to free drug and the targeted nanotherapy (Fig 3, A and B). However, LUVa cells and primary human mast cells both displayed enhanced killing by anti-Sig6-NPmidoFabs compared to free midostaurin ($P < .0001$ for both), demonstrating a half-maximal inhibitory concentration of 0.6047 $\mu\text{g/mL}$ in LUVa cells and 0.4178 $\mu\text{g/mL}$ in human primary mast cells (Fig 3, C and D).

Differential Siglec-6 receptor density may account for the differing sensitivity of these cell lines to the targeted nanotherapy. While each of these mast cell lines is capable of degranulation, it is not uncommon for modified or immortalized cell lines to have altered protein expression leading to artifacts unique to cell culture experiments.^{56,66,67} We utilized Quantum Simply Cellular beads to quantify Siglec-6 receptor density by flow cytometry.⁴⁶ Both ROSA cell lines express the lowest amount of Siglec-6, comparable to each other—around 5,000 receptors per cell. In comparison, LUVa cells expressed around 12,000 receptors per cell (see Fig E1 in the Online Repository available at www.jacionline.org).

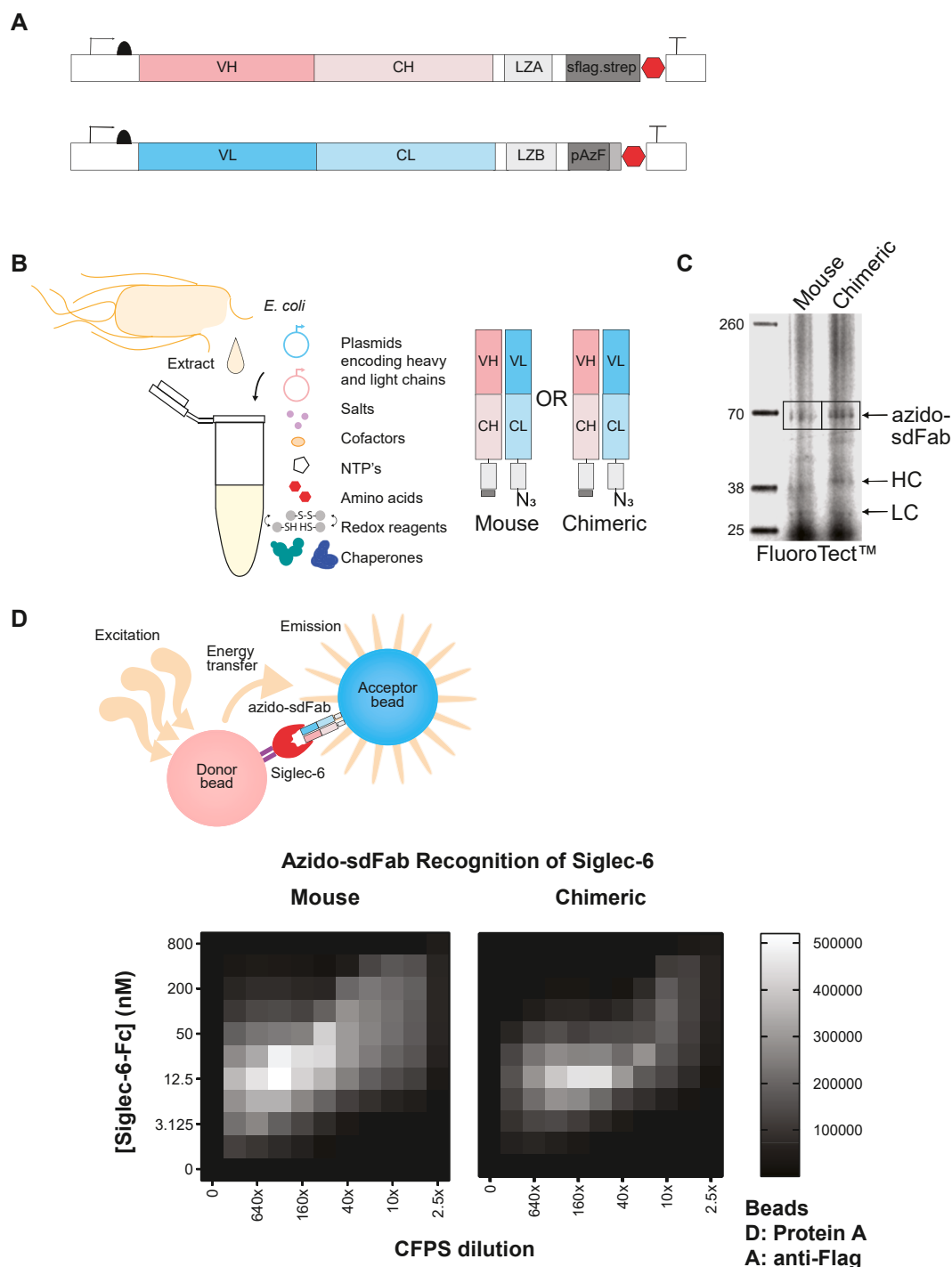


FIG 1. Design and production of anti-Siglec-6 targeting molecules (azido-sdFabs). **(A)** Azido-sdFabs are designed as separate heavy- and light-chain constructs with downstream leucine zipper domain for synthetic dimerization (sd), affinity tags, and site for azido-modified phenylalanine incorporation for click chemistry conjugation (azido). **(B)** Constructs are encoded on 2 separate plasmids and supplemented into cell-free protein synthesis (CFPS) reactions along with necessary building blocks, substrates, and reagents to create oxidizing environment for assembly. **(C)** Incorporation of fluorescently labeled lysine enables visualization of fully assembled azido-sdFab observed at ~70 kDa with unassembled chains visible at lower masses. **(D)** *In vitro* binding is detected by in-solution, bead-based binding assay (AlphaLISA) where cotitration of Siglec-6 (y-axis) and azido-sdFabs (x-axis) exhibits typical signal pattern indicative of successful Siglec-6 binding for both mouse and chimeric anti-Siglec-6 azido-sdFabs. Reflective of 3 experiments.

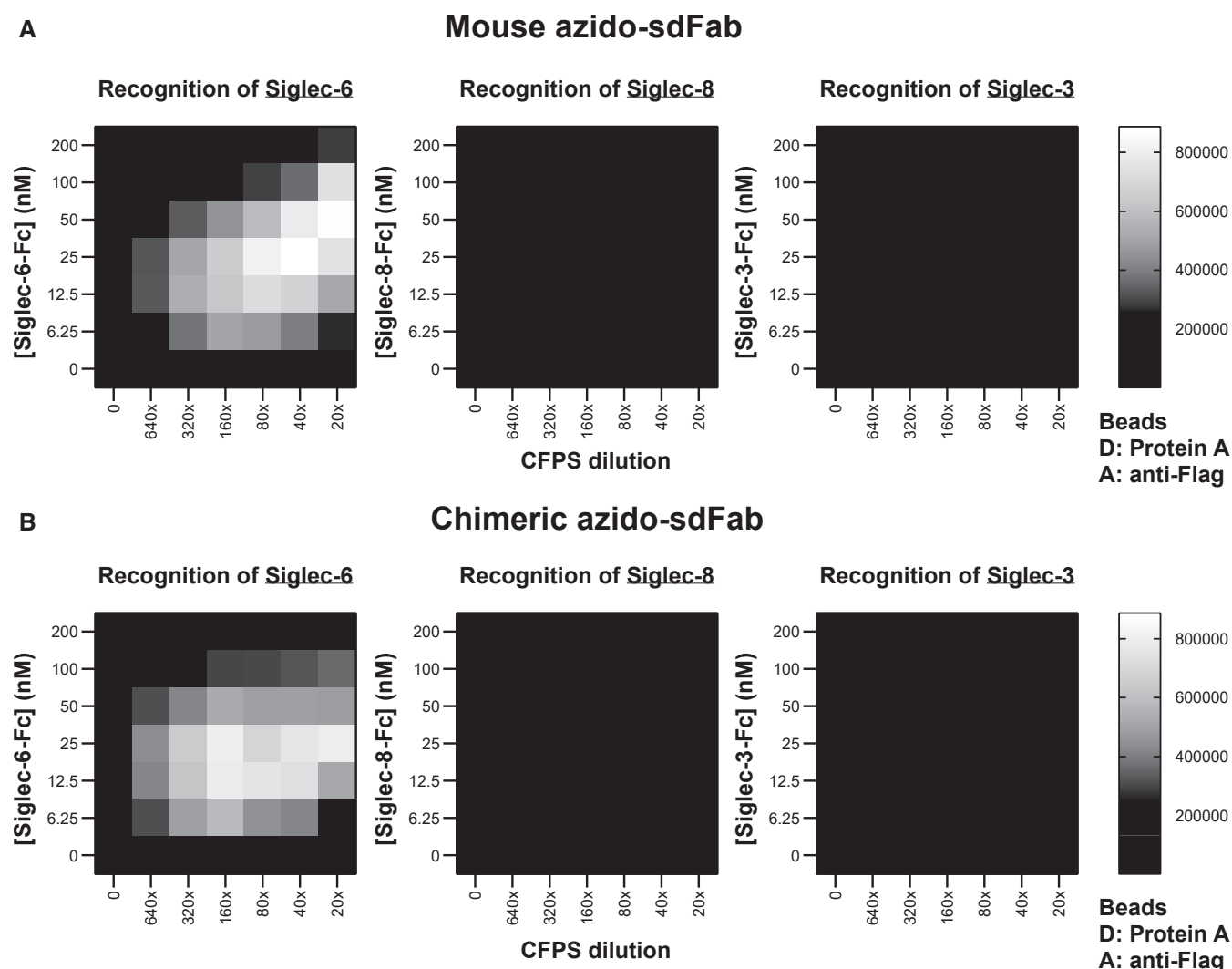


FIG 2. Anti-Siglec-6 azido-sdFab selectivity. **(A)** Mouse anti-Siglec-6 azido-sdFabs demonstrate binding by AlphaLISA assay to Siglec-6, but not to Siglec-8 or Siglec-3 (CD33). **(B)** Chimeric anti-Siglec-6 azido-sdFabs demonstrate binding to Siglec-6, but not to Siglec-8 or Siglec-3. Both versions exhibit desired selectivity with similar affinity for Siglec-6.

jacionline.org). In a previous characterization of Siglec-6 on primary human skin mast cells, we observed about 100,000 receptors per cell.⁴⁶ Receptor expression was sampled from 3 time points between different passages, and the number of Siglec-6 receptors was stable for each cell type.

Generation of a Siglec-6 KI mouse model

Because there is no mouse ortholog of Siglec-6, a Siglec-6 KI mouse model was generated by using a similar strategy to that previously deployed to generate Siglec-8 mast cell-KI mice.⁶⁸ Transgenic mice expressing human SIGLEC6 cDNA in a Cre-inducible manner (SIGLEC6^{LSL}) were crossed with strains expressing Cre under control of the promoter for the mast cell protease carboxypeptidase A3 (Cpa3). This achieves mast cell-selective expression of Siglec-6 and provides an *in vivo* model (Siglec6Cpa3) for assessing the effect of targeting Siglec-6 on mast cells (Fig 4, A, and see Fig E2 in the Online Repository available at www.jacionline.org). In this mouse model, nearly all mast

cells express Siglec-6 at a density of about 100,000 receptors per cell, which is comparable to that observed on primary human skin mast cells. Very weak Siglec-6 signal is detected in a subset of CD11b⁺ and Ly6G⁺ cells, which may indicate Siglec-6 expression in a small number of neutrophils as well (Fig 4, B). At baseline, no differences are observed between Siglec-6 KI, Siglec-8 KI, or C57BL/6 mice in mast cell responses in a passive systemic anaphylaxis model or mast cell numbers (see Fig E3 in the Online Repository).

Anti-Sig6-NPmidoFabs selectively eliminate mast cells in Siglec-6 KI mice

To evaluate selective mast cell elimination *in vivo*, Siglec-6 KI mice were administered anti-Sig6-NPmidoFabs via intraperitoneal injection (Fig 5, A). Leukocyte common antigen (CD45)-positive cells from peritoneal lavage samples were detected and analyzed by flow cytometry. To identify mast cell-specific elimination, CD45⁺ cells were gated by a combination of Siglec-6,

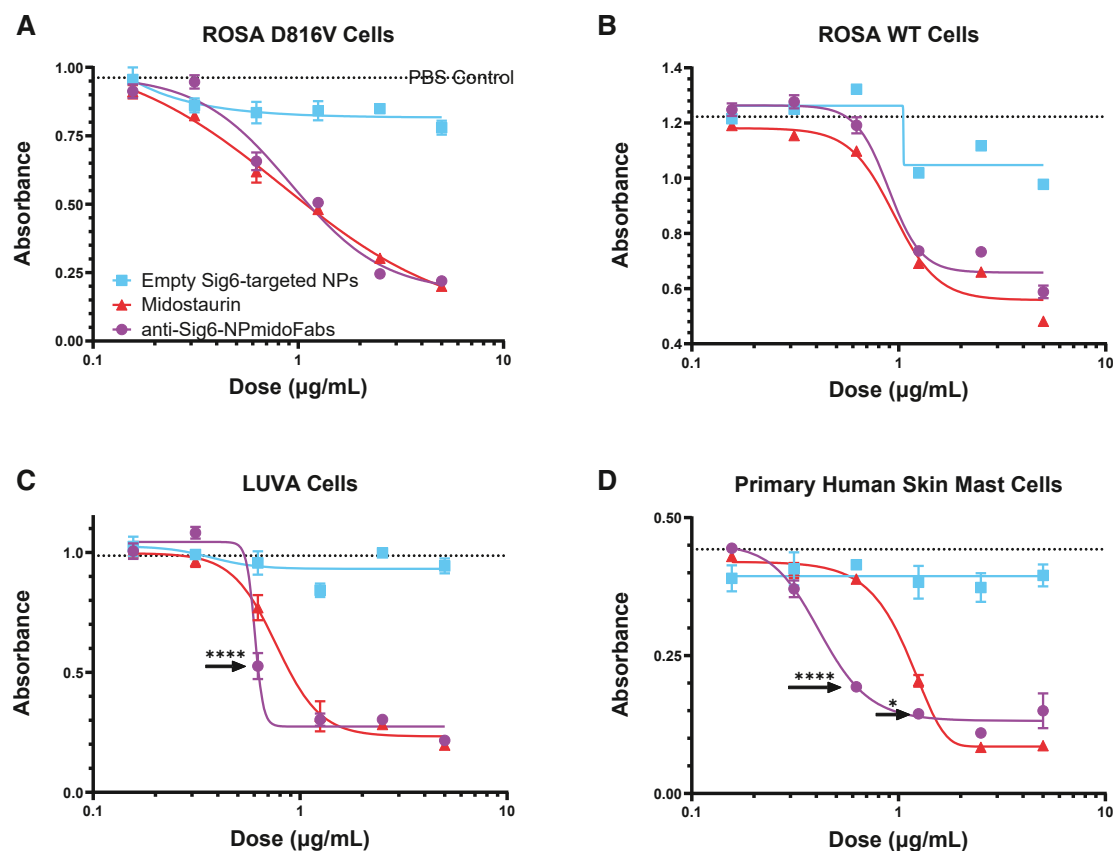


FIG 3. Cell viability assays of anti-Sig6-NPmidoFab-treated human mast cell lines and primary human mast cells. Higher absorbance values correlate to greater cell viability and populations. Anti-Sig6-NPmidoFab elimination of (A) ROSA D816V and (B) ROSA WT cells was comparable to that of free midostaurin. Alternatively, anti-Sig6-NPmidoFab elimination of (C) LUVA cells and (D) primary human skin mast cells, which have higher Siglec-6 receptor density, was more effective to that of free midostaurin. Empty Siglec-6-targeted micelles without drug payload (empty Sig6-targeted NPs) have little to no effect. Significance determined by 2-way ANOVA with multiple comparisons; $n = 3$. * $P < .05$, **** $P < .001$. Half-maximal inhibitory concentration (IC_{50}) values for anti-Sig6-NPmidoFabs vs free drug were, respectively, 0.92 vs 0.85 $\mu\text{g/mL}$ (A), 0.89 vs 0.95 $\mu\text{g/mL}$ (B), 0.6 vs 0.77 $\mu\text{g/mL}$ (C), and 0.42 vs 1.1 $\mu\text{g/mL}$ (D).

KIT, and Fc ϵ RI α expression. CD45⁺ cells without a mast cell marker were used to quantify off-target, non-mast cell toxicity. We observed very strong (95.5%) elimination of mast cells by anti-Sig6-NPmidoFabs compared to free midostaurin or midostaurin-loaded nanocarriers without Siglec-6 targeting (untargeted midostaurin NPs) (46.9%, $P = .005$ and 57.2%, $P = .04$, respectively), and no elimination with empty Siglec-6 targeted nanocarriers (empty Sig6-targeted NPs) ($P = .93$) (Fig 5, B). We also saw a reduction in non-mast cell toxicity among other CD45⁺ cells by anti-Sig6-NPmidoFabs (4.8% off-target elimination) compared to free drug (26.0%, $P = .0002$) or untargeted midostaurin NPs (18.1%, $P = .006$) (Fig 5, C). Additionally, we found that levels of the mucosal mast cell protease mMCP-1 were significantly reduced in the small intestine by 48 hours after administration of the anti-Sig6-NPmidoFabs, indicating that mast cells were likewise depleted in tissues (see Fig E4 in the Online Repository available at www.jacionline.org). No significant alterations of intestinal mMCP-1 levels resulted from treatment with free midostaurin, untargeted midostaurin-loaded nanocarriers, or empty Siglec-6-targeted nanocarriers. We similarly observed selective elimination of Siglec-6⁺ mast cells with

preservation of CD45⁺Siglec-6⁻ cells in a small pilot study with a humanized mast cell malignancy mouse model, where NSG-SGM3 mice were engrafted with ROSA D816V cells and treated with anti-Sig6-NPmidoFabs or free midostaurin (see Fig E5 in the Online Repository).

DISCUSSION

Here we introduce a therapeutic construct, NPmidoFab, enabled by the application of a CFPS platform for generating clickable, synthetically dimerizing Fab fragments (azido-sdFabs), which endow cell targeting capability to nanocarriers. To our knowledge, the combination of features deployed by our platform has not been produced in a single protein before. We base our azido-sdFab specificity on an antibody known to be rapidly internalized, thereby enabling intracellular delivery of the drug payload.⁵⁸ By using midostaurin as the payload and using anti-Siglec-6 azido-sdFabs to selectively target mast cells, we generate a formulation, anti-Sig6-NPmidoFab, as a potential strategy to selectively deliver drug payloads into mast cells while also showing that the construct without payload has no detectable effect on mast

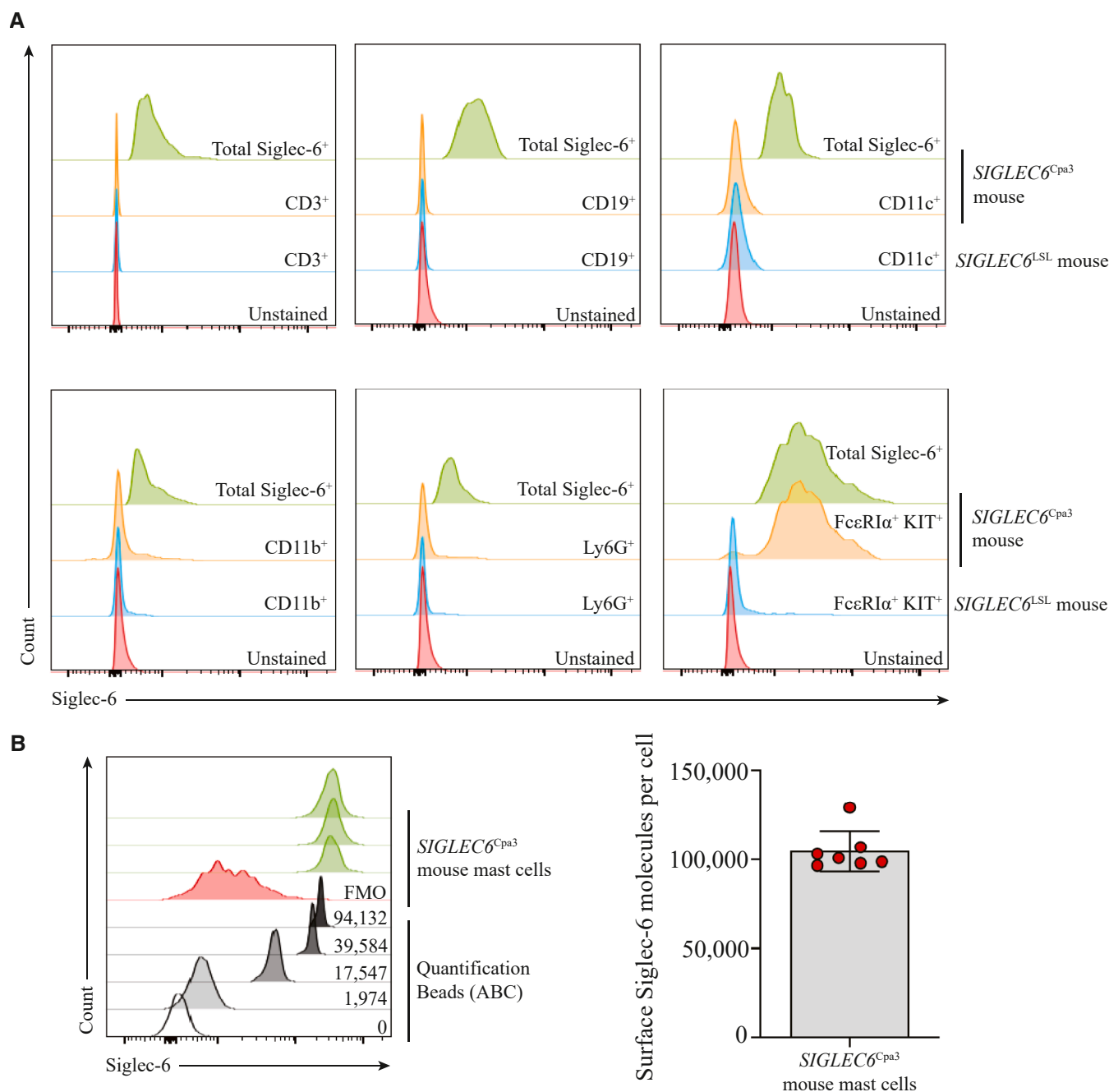


FIG 4. Characterization of Siglec-6 KI mouse model. *SIGLEC6^{LSL}* refers to mouse strain in which human *SIGLEC6* cDNA is present but transcriptionally inactive, as it requires Cre expression to remove floxed STOP cassette. *SIGLEC6^{Cpa3}* refers to mouse in which human *SIGLEC6* is present and Cre recombinase is expressed under control of mast cell-selective *Cpa3* promoter, leading to mast cell-selective Siglec-6 expression. **(A)** Analysis of cell-surface expression of Siglec-6 on mouse leukocytes according to marker expression. Mouse leukocytes from *SIGLEC6^{LSL}* and *SIGLEC6^{Cpa3}* mice were stained for cell type markers CD3, CD19, CD11c, CD11b, Ly6G, FcεRIα, and Kit. Siglec-6 expression in cells bearing indicated marker is compared under Siglec-6⁺ population in same sample (total Siglec-6⁺). Histograms are representative of data from 3 *SIGLEC6^{LSL}* and 3 *SIGLEC6^{Cpa3}* mice. **(B)** Quantification of Siglec-6 receptor expression on mouse mast cells using flow cytometry quantification beads. Histogram demonstrates representative data from 3 *SIGLEC6^{Cpa3}* mice. Antibody binding capacities (ABC) of quantification bead populations are shown. Quantified data include mean, standard deviation, and individual values of 7 *SIGLEC6^{Cpa3}* mice. *Cpa3*, Cre expression driven by carboxypeptidase A3 promoter (permitting Siglec-6 expression); *FMO*, fluorescence-minus-one staining control; *LSL*, loxP-STOP-loxP cassette preventing Siglec-6 expression.

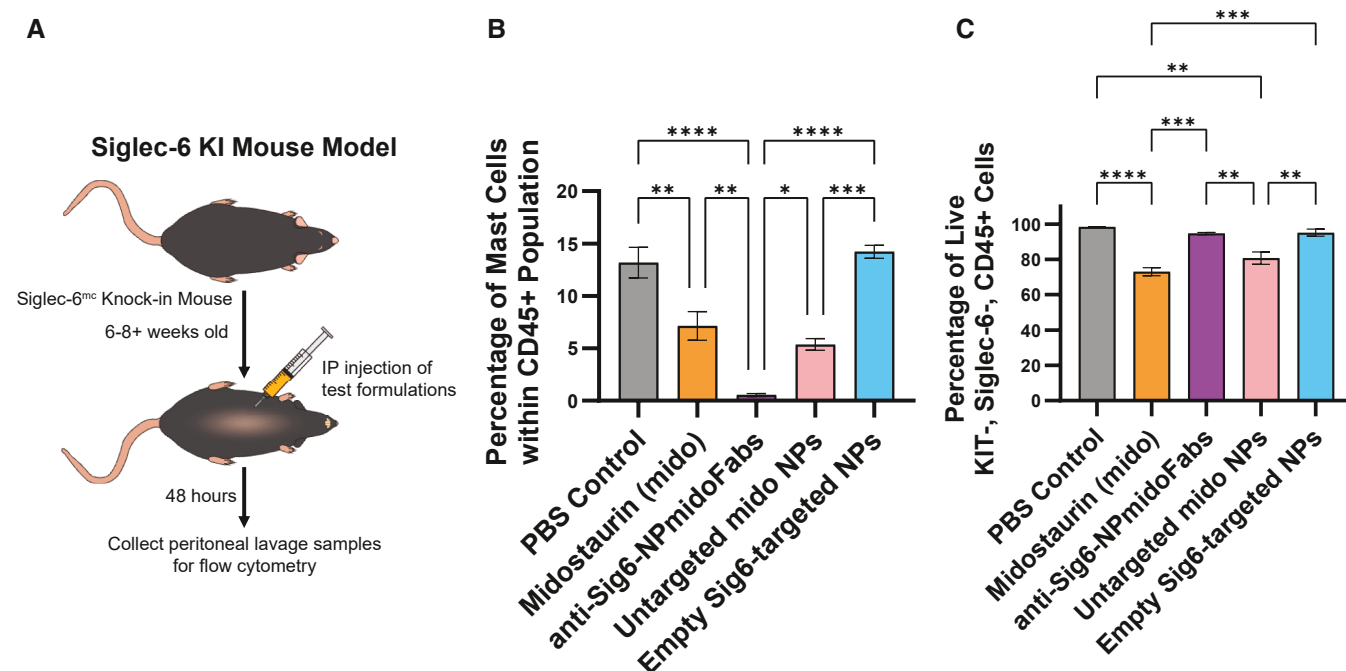


FIG 5. Anti-Sig6-NPmidoFabs more effectively eliminate mast cells with reduced off-target effects in Siglec-6 KI mice. **(A)** Mice received 3.132 μ g dose of midostaurin whether administered as free drug or loaded into nanocarrier. Dose of empty NPs was normalized to particle polymer concentration. After treatment, peritoneal lavage samples from Siglec-6 KI mice were taken and analyzed by flow cytometry. **(B)** Mast cells were effectively eliminated by anti-Sig6-NPmidoFabs (purple bar). Current standard free midostaurin (mido) (yellow bar) and untargeted midostaurin-loaded NPs (untargeted mido NPs) (pink bar) both performed similarly but were 7- to 8-fold less effective at depleting mast cells than anti-Sig6-NPmidoFabs. Empty anti-Siglec-6-targeted NPs without midostaurin (empty Sig6-targeted NPs) and PBS vehicle both displayed no change in mast cell population. **(C)** Changes in non-mast cell CD45⁺ populations were used to measure off-target effects on other immune cells in peritoneal cavity. Off-target effects of anti-Sig6-NPmidoFabs were 4- to 5-fold less than free mido and untargeted mido NPs. Statistical analysis was conducted by 2-way ANOVA with multiple comparisons; $n = 3$, * $P < .05$, ** $P < .01$, *** $P < .005$, **** $P < .001$.

cell viability. We also demonstrate selective elimination of mast cells with reduced off-target effects in several relevant disease models, including a Siglec-6 KI mouse model. Thus, this construct overcomes the off-target effects of the current standard of care, TKIs. These findings provide proof of concept for the potential use of anti-Sig6-NPmidoFab in the treatment of mast cell malignancies, including mastocytosis and mast cell leukemia.

Optimization of the construct is needed for it to be ready for human use. The mouse-human chimeric azido-sdFab retained binding affinity for Siglec-6 compared to its fully mouse counterpart—presumably in the nanomolar range, according to studies correlating flow cytometry antibody performance and formal affinity studies.⁶⁹ However, ideally, azido-sdFabs would be completely humanized before use in human patients.⁷⁰ Also, the half-life of related therapeutics with PEG-b-PPS have been reported to range from days to weeks.^{31,71,72} Further iterations would need to optimize PEG-b-PPS morphology for stability, particularly because vesicles have been shown to be more stable *in vivo*.⁷³ Optimizing azido-sdFab packing while preserving particle integrity would also lead to a more effective construct. Finally, an intravenous route of administration would be desired for ease of patient use. All of these parameters would heavily influence biodistribution and half-life of the therapeutic construct.^{74,75} We demonstrate mast cell depletion in different tissue compartments, but formal biodistribution, time course, and

dose-response testing, as well as further characterization of binding affinity and off-target effects, would best follow construct optimization. Existing studies have shown that inhibition of anaphylaxis is a functional consequence of mast cell depletion, but confirmatory functional testing would also be needed once the construct is optimized to better characterize its effects.⁵⁸

In our models, the observation that human mast cells and cell lines with higher density of surface Siglec-6 expression has increased sensitivity to the therapeutic construct leads to several interesting potential conclusions. First, it supports a Siglec-6-directed mechanism of drug delivery. Second, it emphasizes the dependence of the therapeutic construct, in its current form, on Siglec-6 expression. Fortunately, Siglec-6 expression has been shown to be stable and consistent at $\sim 100,000$ receptors per mast cell across human samples and is similar between allergic and nonallergic patients and patients with and without systemic mastocytosis.^{46,67}

While we formulated anti-Sig6-NPmidoFab with a payload effective for mast cell malignancies, the construct has the potential to be used for nonmalignant mast cell-mediated disease as well. Alternative cargoes such as Bruton TKIs, which have shown efficacy in preventing anaphylaxis, could be loaded into the nanocarrier for improved mast cell-selective delivery.^{76,77} Also, although targeting mast cells would be sufficient to treat chronic spontaneous urticaria and mastocytosis, broadening the

azido-sdFab specificity to also target eosinophils and basophils would allow the construct to also be used to treat other chronic allergic diseases.^{78,79} Given recent findings of Siglec-6 expression on some hematologic malignancies, this targeted nanocarrier formulation may also be useful in the treatment of chronic lymphocytic leukemia, chronic myeloid leukemia, and acute myeloid leukemia if delivering a different chemotherapeutic payload.^{57,67,80} Future NPfab formulations could offer new treatments for a wide range of disorders simply by swapping the cargo and/or azido-sdFab binding specificity. Because of the modularity of the azido-sdFab CFPS platform, this therapeutic construct could conceivably be used to deliver a desired payload to any cell with a surface marker that could be targeted by an antibody, which would extensively broaden the potential applications of this technology.

DISCLOSURE STATEMENT

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Disclosure of potential conflict of interest: A. Helms Thames, C. H. Rische, M. C. Jewett, E. A. Scott, B. S. Bochner, and J. A. O'Sullivan have filed invention disclosures based on the work presented here. B. S. Bochner receives remuneration for serving on the scientific advisory board of Allakos and owns stock in Allakos, of which he is cofounder (which makes him subject to certain restrictions under university policy); receives consulting fees from Third Harmonic Bio and AbbVie; receives publication-related royalty payments from Elsevier and UpToDate; and is coinventor on existing Siglec-8-related patents, and thus may be entitled to a share of royalties received by Johns Hopkins University during development and potential sales of such products, with the terms of this arrangement being managed by Johns Hopkins University and Northwestern University in accordance with their conflict-of-interest policies. M. C. Jewett, whose interests are reviewed and managed by Northwestern University and Stanford University in accordance with their competing interest policies, has financial interest in Pearl Bio, Synolo Therapeutics, Ridge Bio, and Gauntlet Bio. The rest of the authors declare that they have no relevant conflicts of interest.

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Key messages

- A novel cell-free protein synthesis platform enables the production of specialized targeting molecules which are azido-modified, synthetically dimerized Fab fragments that can be conjugated to the surface of NPs.
- There is no mouse ortholog for Siglec-6, so a KI mouse model with mast cell-selective Siglec-6 expression is introduced.
- Formulating the azido-sdFabs into a therapeutic construct where NPs are targeted to Siglec-6 and loaded with midostaurin (anti-Sig6-NPmidoFab) achieves selective mast cell elimination and reduced off-target effects in human mast cell lines, primary human mast cells, and mouse models, offering a new strategy for treating mast cell malignancies with the potential for broad application.

REFERENCES

- Bergstrom A, Hagglund H, Berglund A, Nilsson G, Lambe M. Epidemiology of mastocytosis: a population-based study (Sweden). *Acta Oncol* 2024;63:44-50.
- Gotlib J, Kluin-Nelemans HC, George TI, Akin C, Sotlar K, Hermine O, et al. Efficacy and safety of midostaurin in advanced systemic mastocytosis. *N Engl J Med* 2016;374:2530-41.
- Theoharides TC, Valent P, Akin C. Mast cells, mastocytosis, and related disorders. *N Engl J Med* 2015;373:163-72.
- Valent P, Sperr WR, Akin C. How I treat patients with advanced systemic mastocytosis. *Blood* 2010;116:5812-7.
- Mounie M, Burel HD, Molinier L, Costa N, Livideanu CB. Lifetime disability-adjusted life-year assessment of indolent systemic mastocytosis. *J Allergy Clin Immunol Pract* 2025;13:923-9.
- Galli SJ, Tsai M, Wershil BK. The c-kit receptor, stem cell factor, and mast cells: what each is teaching us about the others. *Am J Pathol* 1993;142:965-74.
- Vosseller K, Stella G, Yee NS, Besmer P. c-Kit receptor signaling through its phosphatidylinositol-3'-kinase-binding site and protein kinase C: role in mast cell enhancement of degranulation, adhesion, and membrane ruffling. *Mol Biol Cell* 1997;8:909-22.
- Akin C, Arock M, Valent P. Tyrosine kinase inhibitors for the treatment of indolent systemic mastocytosis: are we there yet? *J Allergy Clin Immunol* 2022;149:1912-8.
- Valent P, Akin C, Hartmann K, George TI, Sotlar K, Peter B, et al. Midostaurin: a magic bullet that blocks mast cell expansion and activation. *Ann Oncol* 2017;28:2367-76.
- Chandesris M-O, Damaj G, Canioni D, Brouzes C, Lhermitte L, Hanssens K, et al. Midostaurin in advanced systemic mastocytosis. *N Engl J Med* 2016;374:2605-6.
- He H, Tran P, Gu H, Tedesco V, Zhang J, Lin W, et al. Midostaurin, a novel protein kinase inhibitor for the treatment of acute myelogenous leukemia: insights from human absorption, metabolism, and excretion studies of a BDDCS II drug. *Drug Metab Dispos* 2017;45:540-55.
- Stone RM, Manley PW, Larson RA, Capdeville R. Midostaurin: its odyssey from discovery to approval for treating acute myeloid leukemia and advanced systemic mastocytosis. *Blood Adv* 2018;2:444-53.
- Cerny-Reiterer S, Rabenhorst A, Stefanzi G, Herndlhofer S, Hoermann Gregor, Müllauer L, et al. Long-term treatment with imatinib results in profound mast cell deficiency in Ph⁺ chronic myeloid leukemia. *Oncotarget* 2014;6.
- Dhillon S. Avapritinib: first approval. *Drugs* 2020;80:433-9.
- Guarnieri A, Chiccarelli M, Cable L, Bouhana K, Sullivan F, Ball H, et al. Preclinical data with KIT D816V inhibitor bezuclastinib (CGT9486) demonstrates high selectivity and minimal brain penetrance. *Blood* 2021;138(suppl 1):4595.
- Des Soye BJ, Gerbasi VR, Thomas PM, Kelleher NL, Jewett MC. A highly productive, one-pot cell-free protein synthesis platform based on genomically recoded *Escherichia coli*. *Cell Chem Biol* 2019;26:1743-54.e9.
- DeWinter MA, Thames AH, Guerrero L, Kightlinger W, Karim AS, Jewett MC. Point-of-care peptide hormone production enabled by cell-free protein synthesis. *ACS Synth Biol* 2023;12:1216-26.
- Hershowe J, Kightlinger W, Jewett MC. Cell-free systems for accelerating glycoprotein expression and biomanufacturing. *J Ind Microbiol Biotechnol* 2020;47:977-91.

19. Hunt AC, Case JB, Park YJ, Cao L, Wu K, Walls AC, et al. Multivalent designed proteins neutralize SARS-CoV-2 variants of concern and confer protection against infection in mice. *Sci Transl Med* 2022;14(646):eabn1252.
20. Jaroentomeechai T, Stark JC, Natarajan A, Glasscock CJ, Yates LE, Hsu KJ, et al. Single-pot glycoprotein biosynthesis using a cell-free transcription-translation system enriched with glycosylation machinery. *Nat Commun* 2018;9:2686.
21. Kightlinger W, Duncker KE, Ramesh A, Thames AH, Natarajan A, Stark JC, et al. A cell-free biosynthesis platform for modular construction of protein glycosylation pathways. *Nat Commun* 2019;10:5404.
22. Warfel KF, Williams A, Wong DA, Sobol SE, Desai P, Li J, et al. A low-cost, thermostable, cell-free protein synthesis platform for on-demand production of conjugate vaccines. *ACS Synth Biol* 2023;12:95-107.
23. Thames AH, Moons SJ, Wong DA, Boltje TJ, Bochner BS, Jewett MC. GlycoCAP: a cell-free, bacterial glycosylation platform for building clickable azido-sialoglycoproteins. *ACS Synth Biol* 2023;12:1264-74.
24. Thames AH, Rische CH, Cao Y, Krier-Burris RA, Kuang FL, Hamilton RG, et al. A cell-free protein synthesis platform to produce a clinically relevant allergen panel. *ACS Synth Biol* 2023;12:2252-61.
25. Hunt AC, Rasor BJ, Seki K, Ekas HM, Warfel KF, Karim AS, et al. Cell-free gene expression: methods and applications. *Chem Rev* 2025;125:91-149.
26. Silverman AD, Karim AS, Jewett MC. Cell-free gene expression: an expanded repertoire of applications. *Nat Rev Genet* 2020;21:151-70.
27. Kolb HC, Finn MG, Sharpless KB. Click chemistry: diverse chemical function from a few good reactions. *Angew Chem Intl Ed* 2001;40:2004-21.
28. Baskin JM, Bertozzi CR. Bioorthogonal click chemistry: covalent labeling in living systems. *QSAR and Combinatorial Science* 2007;26:1211-9.
29. Baskin JM, Prescher JA, Laughlin ST, Agard NJ, Chang PV, Miller IA, et al. Copper-free click chemistry for dynamic *in vivo* imaging. *Proc Natl Acad Sci U S A* 2007;104:16793-7.
30. Martin RW, Des Soye BJ, Kwon YC, Kay J, Davis RG, Thomas PM, et al. Cell-free protein synthesis from genomically recoded bacteria enables multisite incorporation of noncanonical amino acids. *Nat Commun* 2018;9:1203.
31. Allen SD, Liu YG, Kim T, Bobbala S, Yi S, Zhang X, et al. Celastrol-loaded PEG-b-PPS nanocarriers as an anti-inflammatory treatment for atherosclerosis. *Biomater Sci* 2019;7:657-68.
32. Nishiyama N, Kataoka K. Nanostructured devices based on block copolymer assemblies for drug delivery: designing structures for enhanced drug function. In: Satchi-Fainaro R, Duncan R, editors. *Polymer therapeutics II*. New York: Springer Nature; 2006. pp. 67-101.
33. Kataoka K, Harada A, Nagasaki Y. Block copolymer micelles for drug delivery: design, characterization and biological significance. *Adv Drug Deliv Rev* 2001;47:113-31.
34. Velluto D, Demurta S D, Hubbell JA. PEG-b-PPS diblock copolymer aggregates for hydrophobic drug solubilization and release: cyclosporin A as an example. *Mol Pharm* 2008;5:632-42.
35. Yi S, Karabin NB, Zhu J, Bobbala S, Lyu H, Li S, et al. An injectable hydrogel platform for sustained delivery of anti-inflammatory nanocarriers and induction of regulatory T cells in atherosclerosis. *Front Bioeng Biotechnol* 2020;8:542.
36. Alvarado D, Maurer M, Gedrich R, Seibel SB, Murphy MB, Crew L, et al. Anti-KIT monoclonal antibody CDX-0159 induces profound and durable mast cell suppression in a healthy volunteer study. *Allergy* 2022;77:2393-403.
37. Ashman LK. The biology of stem cell factor and its receptor c-Kit. *Int J Biochem Cell Biol* 1999;31:1037-51.
38. Botchkareva NV, Khlgtian M, Jack Longley B, Botchkarev VA, Gilchrist BA. SCF/c-kit signaling is required for cyclic regeneration of the hair pigmentation unit. *FASEB J* 2001;15:645-58.
39. Broudy VC. Stem cell factor and hematopoiesis. *Blood* 1997;90:1345-64.
40. Cardoso HJ, Figueira MI, Correia S, Vaz CV, Socorro S. The SCF/c-KIT system in the male: survival strategies in fertility and cancer. *Mol Reprod Dev* 2014;81:1064-79.
41. Choo E, Dando R. The c-kit receptor tyrosine kinase marks sweet or umami sensing T1R3 positive adult taste cells in mice. *Chemoreception Perception* 2021;14:41-6.
42. Frumento G, Zuo J, Verma K, Croft W, Ramagiri P, Chen FE, et al. CD117 (c-Kit) is expressed during CD8⁺ T cell priming and stratifies sensitivity to apoptosis according to strength of TCR engagement. *Front Immunol* 2019;10:468.
43. Lammie A, Drobnjak M, Gerald W, Saad A, Cote R, Cordon-Cardo C. Expression of c-kit and kit ligand proteins in normal human tissues. *J Histochem Cytochem* 1994;42:1417-25.
44. Metz M, Maurer M, Arnau AMG, Hussen N, Staikūnienė-Kozonis J, Slomskis T, et al. Treatment with barzolvolimab improves urticaria control and quality of life in patients with chronic inducible urticaria: phase 2 12 week results. *J Allergy Clin Immunol* 2025;155:AB59.
45. Rambhatla A, Strug MR, De Paredes JG, Cordoba Munoz MI, Thakur M. Fertility considerations in targeted biologic therapy with tyrosine kinase inhibitors: a review. *J Assist Reprod Genet* 2021;38:1897-908.
46. Robida PA, Rische CH, Morgenstern NB, Janarthanam R, Cao Y, Krier-Burris RA, et al. Functional and phenotypic characterization of Siglec-6 on human mast cells. *Cells* 2022;11:1138.
47. Mizrahi S, Gibbs BF, Karra L, Ben-Zimra M, Levi-Schaffer F. Siglec-7 is an inhibitory receptor on human mast cells and basophils. *J Allergy Clin Immunol* 2014;134:230-3.
48. Kikly KK, Bochner BS, Freeman SD, Tan KB, Gallagher KT, D'Alessio KJ, et al. Identification of SAF-2, a novel Siglec expressed on eosinophils, mast cells, and basophils. *J Allergy Clin Immunol* 2000;105(6 pt 1):1093-100.
49. Hudson SA, Herrmann H, Du J, Cox P, Haddad el B, Butler B, et al. Developmental, malignancy-related, and cross-species analysis of eosinophil, mast cell, and basophil Siglec-8 expression. *J Clin Immunol* 2011;31:1045-53.
50. Miralda I, Samanas NB, Seo AJ, Foronda JS, Sachen J, Hui Y, et al. Siglec-9 is an inhibitory receptor on human mast cells *in vitro*. *J Allergy Clin Immunol* 2023;152:711-24.e14.
51. O'Sullivan JA, Chang AT, Youngblood BA, Bochner BS. Eosinophil and mast cell Siglecs: from biology to drug target. *J Leukoc Biol* 2020;108:73-81.
52. Amadori S, Suciu S, Selleslag D, Aversa F, Gaidano G, Musso M, et al. Gemtuzumab ozogamicin versus best supportive care in older patients with newly diagnosed acute myeloid leukemia unsuitable for intensive chemotherapy: results of the randomized phase III EORTC-GIMEMA AML-19 trial. *J Clin Oncol* 2016;34:972-9.
53. O'Sullivan JA, Carroll DJ, Cao Y, Salicru AN, Bochner BS. Leveraging Siglec-8 endocytic mechanisms to kill human eosinophils and malignant mast cells. *J Allergy Clin Immunol* 2018;141:1774-85.e7.
54. Dellon ES, Peterson KA, Murray JA, Falk GW, Gonsalves N, Chehade M, et al. Anti-Siglec-8 antibody for eosinophilic gastritis and duodenitis. *N Engl J Med* 2020;383:1624-34.
55. O'Sullivan JA, Youngblood BA, Schleimer RP, Bochner BS. Siglecs as potential targets of therapy in human mast cell- and/or eosinophil-associated diseases. *Semin Immunol* 2023;69:101799.
56. Yokoi H, Myers A, Matsumoto K, Crocker PR, Saito H, Bochner BS. Alteration and acquisition of Siglecs during *in vitro* maturation of CD34⁺ progenitors into human mast cells. *Allergy* 2006;61:769-76.
57. Nunes J, Tafesse R, Mao C, Purcell M, Mo X, Zhang L, et al. Siglec-6 as a therapeutic target for cell migration and adhesion in chronic lymphocytic leukemia. *Nat Commun* 2024;15:5180.
58. Schanin J, Korver W, Brock EC, Leung J, Benet Z, Luu T, et al. Discovery of an agonistic Siglec-6 antibody that inhibits and reduces human mast cells. *Commun Biol* 2022;5:1226.
59. Allakos. Allakos announces topline results from its phase 1 trial of AK006 in patients with chronic spontaneous urticaria and announces restructuring. Press release; January 27, 2025. Available at: <https://www.arizonascitechgazette.com/article/780646336-allakos-announces-topline-results-from-its-phase-1-trial-of-ak006-in-patients-with-chronic-spontaneous-urticaria-and-announces-restructuring>.
60. Ojima-Kato T, Fukui K, Yamamoto H, Hashimura D, Miyake S, Hirakawa Y, et al. "Zipbody" leucine zipper-fused Fab in *E coli in vitro* and *in vivo* expression systems. *Protein Eng Des Sel* 2016;29:149-57.
61. Hunt AC, Vogeli B, Hassan AO, Guerrero L, Kightlinger W, Yoesep DJ, et al. A rapid cell-free expression and screening platform for antibody discovery. *Nat Commun* 2023;14:3897.
62. Saleh R, Wedeh G, Herrmann H, Bibi S, Cerny-Reiterer S, Sadovnik I, et al. A new human mast cell line expressing a functional IgE receptor converts to tumorigenic growth by KIT D816V transfection. *Blood* 2014;124:111-20.
63. Laidlaw TM, Steinke JW, Tinana AM, Feng C, Xing W, Lam BK, et al. Characterization of a novel human mast cell line that responds to stem cell factor and expresses functional FcεpsilonRI. *J Allergy Clin Immunol* 2011;127:815-22.e1-5.
64. Kambe N, Kambe M, Kochan JP, Schwartz LB. Human skin-derived mast cells can proliferate while retaining their characteristic functional and protease phenotypes. *Blood* 2001;97:2045-52.
65. Caslin HL, Taruselli MT, Paranjape A, Kiwanuka K, Haque T, Chumanevich AP, et al. The use of human and mouse mast cell and basophil cultures to assess type 2 inflammation. In: Reinhardt RL, editor. *Type 2 immunity: methods and protocols*. New York: Springer; 2018. pp. 81-92.
66. Hermans MAW, van Stigt AC, van de Meerendonk S, Schrijver B, van Daele PLA, van Hagen PM, et al. Human mast cell line HMC1 expresses functional Mas-related G-protein coupled receptor 2. *Front Immunol* 2021;12.
67. Smiljkovic D, Herrmann H, Sadovnik I, Gamperl S, Berger D, Stefanzi G, et al. Expression and regulation of Siglec-6 (CD327) on human mast cells and basophils. *J Allergy Clin Immunol* 2023;151:202-11.

68. Wei Y, Chhiba KD, Zhang F, Ye X, Wang L, Zhang L, et al. Mast Cell-Specific Expression of human Siglec-8 in conditional knock-in mice. *Int J Mol Sci* 2018;20.
69. Geuijen CAW, Clijsters-van der Horst M, Cox F, Rood PML, Throsby M, Jongeneelen MAC, et al. Affinity ranking of antibodies using flow cytometry: application in antibody phage display-based target discovery. *J Immunol Methods* 2005;302:68-77.
70. Lu RM, Hwang YC, Liu IJ, Lee CC, Tsai HZ, Li HJ, et al. Development of therapeutic antibodies for the treatment of diseases. *J Biomed Sci* 2020;27:1.
71. Harris JM, Chess RB. Effect of pegylation on pharmaceuticals. *Nat Rev Drug Discov* 2003;2:214-21.
72. Karabin NB, Allen S, Kwon H-K, Bobbala S, Firlar E, Shokuhfar T, et al. Sustained micellar delivery via inducible transitions in nanostructure morphology. *Nat Commun* 2018;9:624.
73. Photos PJ, Bacakova L, Discher B, Bates FS, Discher DE. Polymer vesicles *in vivo*: correlations with PEG molecular weight. *J Control Release* 2003;90:323-34.
74. Yi S, Allen SD, Liu YG, Ouyang BZ, Li X, Augsornworawat P, et al. Tailoring nanostructure morphology for enhanced targeting of dendritic cells in atherosclerosis. *ACS Nano* 2016;10:11290-303.
75. Vincent MP, Bobbala S, Karabin NB, Frey M, Liu Y, Navidzadeh JO, et al. Surface chemistry-mediated modulation of adsorbed albumin folding state specifies nano-carrier clearance by distinct macrophage subsets. *Nat Commun* 2021;12:648.
76. Dispenza MC, Krier-Burris RA, Chhiba KD, Udem BJ, Robida PA, Bochner BS. Bruton's tyrosine kinase inhibition effectively protects against human IgE-mediated anaphylaxis. *J Clin Invest* 2020;130:4759-70.
77. Suresh RV, Dunnam C, Vaidya D, Wood RA, Bochner BS, MacGlashan DW Jr, et al. A phase II study of Bruton's tyrosine kinase inhibition for the prevention of anaphylaxis. *J Clin Invest* 2023;133.
78. Gorelik M, Narisety SD, Guerrero AL, Chichester KL, Keet CA, Bieneman AP, et al. Suppression of the immunologic response to peanut during immunotherapy is often transient. *J Allergy Clin Immunol* 2015;135:1283-92.
79. Stevens WW, Lee RJ, Schleimer RP, Cohen NA. Chronic rhinosinusitis pathogenesis. *J Allergy Clin Immunol* 2015;136:1442-53.
80. Jetani H, Navarro-Bailón A, Maucher M, Frenz S, Verbruggen C, Yeguas A, et al. Siglec-6 is a novel target for CAR T-cell therapy in acute myeloid leukemia. *Blood* 2021;138:1830-42.