Stable High-Concentration Monoclonal Antibody Formulations Enabled by an Amphiphilic Copolymer Excipient

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Monoclonal antibodies (mAbs) are a staple in modern pharmacotherapy. Unfortunately, these biopharmaceuticals are limited by their tendency to aggregate in formulation, resulting in poor stability and often requiring low concentration drug formulations. Existing excipients designed to stabilize formulations are often limited by their toxicity and tendency to form particles such as micelles. Here, the ability of a simple "drop-in," amphiphilic copolymer excipient to enhance the stability of high concentration formulations of clinically relevant mAbs without altering their pharmacokinetics or injectability is demonstrated. Through interfacial rheology and surface tension measurements, it is demonstrated that the copolymer excipient competitively adsorbs to formulation interfaces. Further, through determination of monomeric composition and retained bioactivity after stressed aging, it is shown that this excipient confers a significant stability benefit to high concentration antibody formulations. Finally, it is demonstrated that the excipient behaves as an inactive ingredient, having no significant impact on the pharmacokinetic profile of a clinically relevant antibody in mice. This amphiphilic copolymer excipient demonstrates promise as an additive to create stable, high concentration antibody formulations, thereby enabling improved treatment options such as a route-of-administration switch from low concentration intravenous (IV) to high concentration subcutaneous (SC) delivery while reducing dependence on the cold chain.

1. Introduction

Biopharmaceuticals are rapidly replacing small organic molecules as the leading active pharmaceutical ingredients (APIs) on the market today.[1] Monoclonal antibodies (mAbs) are one broadly important class of biopharmaceuticals that have been thoroughly investigated for their superior target selectivity and reduced toxicity when compared to small organic molecules. These advantages notwithstanding, many antibody-based therapeutics have been limited in their translational success due to formulation instabilities, most generally aggregation, arising from their complex tertiary structure, large molecular weight, and inherently amphiphilic nature.[2,3] While the mechanism of aggregation varies slightly from antibody to antibody, many contain hydrophobic patches or partially denature to reveal hydrophobic segments of the molecule that drive adsorption of the proteins onto interfaces (e.g., air–water, glass–water, or rubber–water interfaces).[3–5] The high local concentrations of the molecules at these interfaces, coupled with interface-mediated partial

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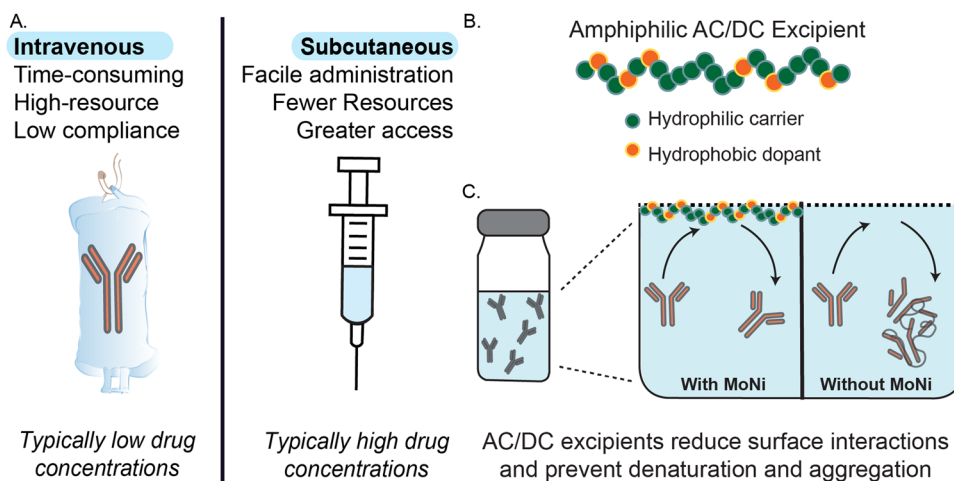LCPO
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Figure 1. Overview of stabilization of high-concentration antibody formulations using novel copolymer excipients. A) Schematic overview of current therapeutic routes of administration for biopharmaceuticals, whereby intravenous administration is common because of limitations on stability of biopharmaceutics at the high concentrations required for subcutaneous administration, resulting in high patient burden. B) Graphical representation of amphiphilic acrylamide carrier/dopant copolymer (AC/DC) excipients, such as lead candidate poly(acryloylmorpholine-*co-N*-isoproylacrylamide) (MoNi), that can be used to stabilize biopharmaceuticals in formulation. C) Proposed mechanism of stabilization of monoclonal antibodies in formulation using AC/DC copolymers as simple "drop-in" excipient. Since AC/DC copolymers are amphiphilic, they preferentially absorb to interfaces, precluding antibody interfacial adsorption in formulation and thereby preventing interface-related destabilization and aggregation events.

unfolding, trigger initial nucleation of aggregation events that lead to further aggregation.[3,5] This tendency to aggregate at interfaces increases with increasing formulation concentration, negatively impacting overall formulation stability. These inherent concentration limitations often require that large-volume, low-concentration transfusions of mAb therapies are delivered intravenously (IV) (**Figure 1**A). Unfortunately, IV administration places a larger burden on patients compared to other routes of administration, requiring lengthy transfusion procedures and access to clinical infrastructure, which at best is inconvenient and at worst could preclude patients from receiving effective treatment. High concentration formulations of mAbs would be necessary to enable dose-matched subcutaneous (SC) injections that are significantly less burdensome on patients and which can also be administered in low resource settings (Figure 1A).[6]

Formulation limitations are particularly detrimental for clinical indications that require maintenance of high mAb serum concentrations for therapeutic benefit. For example, broadly neutralizing HIV antibodies (bnAbs) are a class of antibody therapeutics specifically targeting highly conserved regions on the HIV envelope trimer.[7] HIV bnAbs are currently being explored for their use in prophylactic infection prevention and disease management through passive immunization.[8] While the use of bnAbs for HIV prevention and treatment has been demonstrated in mice and non-human primates,[9] bnAb formulations must typically be administered by IV infusion to reach the high serum concentrations required for protection against breakthrough infection and to prevent onset of drug resistance.[8] According to UNAIDS, there were 1.7 million new annual infections and ≈38 million people living with HIV in 2021; it follows that the development of new therapeutics for the treatment and prevention of HIV that can be deployed even in resource limited settings remains a high priority. SC mAb therapy is a more scalable pathway toward providing prophylactic protection to at-risk popula-

tions and treatment for those currently living with HIV, but as noted, this strategy necessitates the development of stable, high concentration mAb formulations to simplify treatment and improve global access.[6,10,11]

The addition of excipients to biopharmaceutical formulations is a common strategy to overcome challenges associated with high concentration formulation of mAbs, but the number of approved excipients for these formulations is limited.[] Biopharmaceutical formulations commonly contain polyoxyethylene-based surfactants such as polysorbates and poloxamers,[13,14] and commercially, polysorbate 80 and polysorbate 20 are the most commonly used.[12] Several other surfactants have been explored preclinically, but none to date satisfactorily address all criteria for clinical use in parenteral injections.[12] Despite their widespread use, these surfactant systems still suffer several limitations. Many are limited by their critical micelle concentrations (CMCs), the concentration above which the excipient will form micelles, at which point they may further destabilize the formulation by introducing large hydrophobic surfaces.[15] Other mechanisms of toxicity and instability exist; for example, polysorbates degrade through oxidative degradation, resulting in damaging peroxides, and other undesirable interactions between the excipient and the cargo in the bulk may reduce therapeutic activity of the cargo.[15] Further, some polymer excipients have been explored as stabilizers in lyophilization or immobilization, but not for liquid drug formulations as simple drop-in excipients.[16] Interestingly, recent work demonstrates the development of zwitterionic and trehalose polymers for insulin stabilization, but the stressed aging studies are only conducted for 4 h, which is insufficient to evaluate long-term performance, particularly in the context of reducing cold chain reliance.[17] A more translatable approach toward stabilizing biopharmaceuticals would use an excipient that could be easily and stably incorporated into highly concentrated commercial formulations without interacting with the therapeutic

in the bulk, altering its pharmacokinetics, or increasing toxicity while conferring stability over extended time scales.

A promising strategy to stabilize protein formulations is using amphiphilic acrylamide carrier/dopant copolymer (AC/DC) excipients, which preferentially occupy interfaces to prevent interface-mediated protein aggregation. In previous work, we have used this class of excipients to stabilize low molecular weight (<5.8 kDa) biopharmaceuticals such as insulin analogues and pramlintide (an amylin analogue).[18,19] Further, we previously demonstrated that these AC/DC excipients are biocompatible and exhibit low toxicity in both in vitro cytotoxicity assays and in vivo blood chemistry analysis. In a repeated dosing scenario with diabetic rats, no liver or kidney toxicity was observed by common markers.[18,19] However, these excipients have not yet been demonstrated to stabilize mAbs at high concentrations in formulation, where the API is both much larger (150 kDa compared to 5.8 kDa) and formulated at much higher concentrations (>100 mg mL^{-1} compared to 3.5 mg mL^{-1}).

In this study, we utilize a lead-candidate AC/DC excipient, poly(acryloylmorpholine-co-N-isopropylacrylamide) (MoNi), to enable high-concentration formulation of PGT121, a promising anti-HIV bnAb, that is suitable for SC injection. Mechanistically, we investigate the ability of MoNi to preferentially occupy the interfaces in mAb formulations using both interfacial rheology and surface tension measurements. Next, we probe the stability of PGT121 formulations with and without added MoNi using accelerated aging assays at different API concentrations. We then conduct pharmacokinetic studies in mice, comparing SC administration of a low volume of a MoNi-stabilized high-concentration PGT121 formulation to intraperitoneal (IP) administration of a larger volume of a standard low-concentration PGT121 formulation. Overall, we demonstrate that MoNi significantly improves the stability of mAb formulations without altering pharmacokinetics or bioavailability, illustrating its potential as a "drop-in" excipient.

2. Results and Discussion

2.1. Acrylamide Carrier/Dopant Copolymer Excipient Selectively Adsorbs to Interfaces in Monoclonal Antibody Formulations
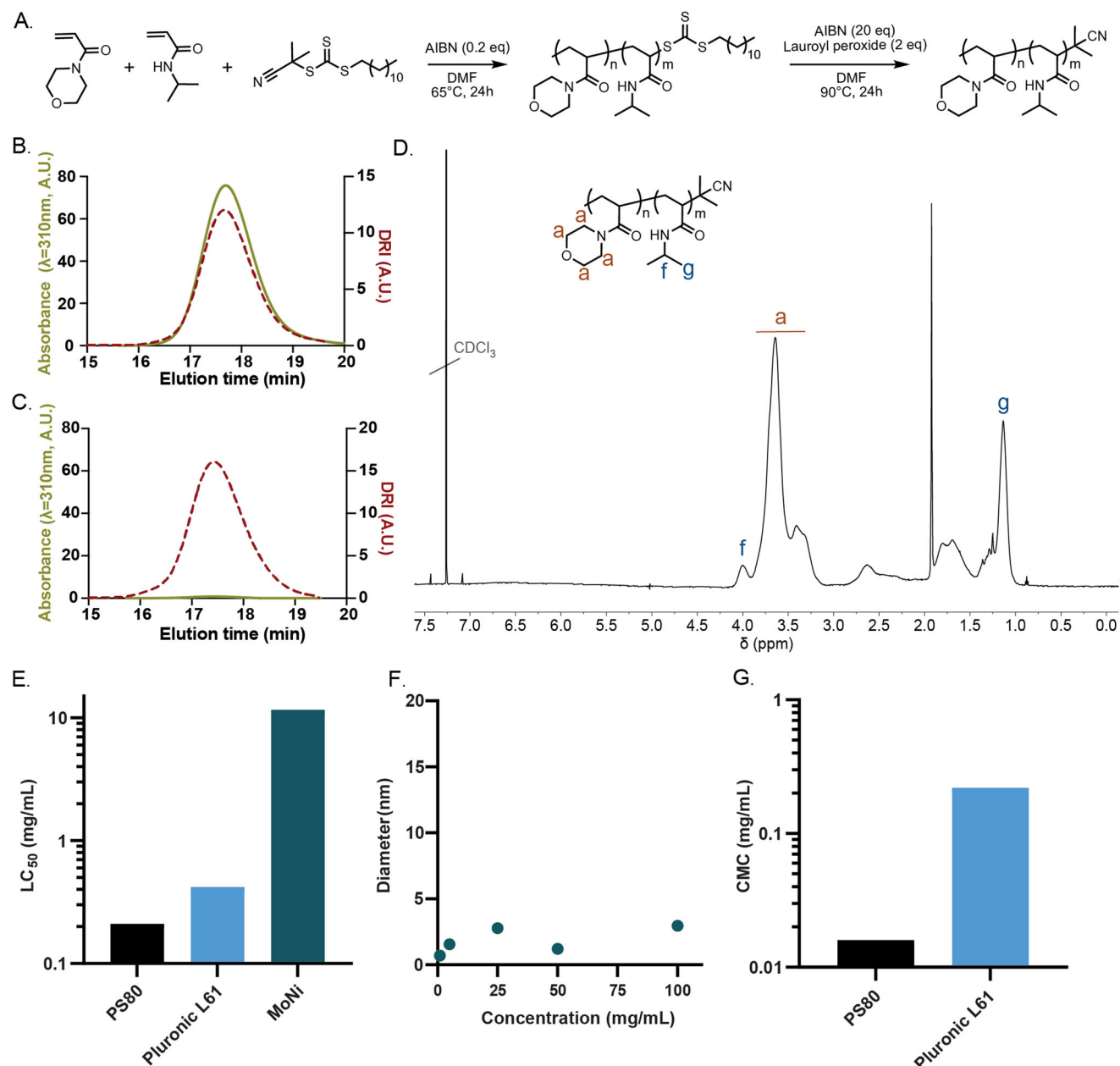
From a previous screen of hundreds of amphiphilic AC/DC copolymers for their ability to stabilize insulin, we identified several top-performing excipient candidates.[18,19] In particular, a copolymer of acryloylmorpholine and N-isopropylacrylamide, referred to as "MoNi," exhibited exceptional ability to stabilize proteins in formulation by preferentially occupying interfaces.[19] We hypothesized that MoNi could similarly impart improved stability to mAb formulations by reducing interface-mediated changes in protein conformation and aggregation-nucleating events, thereby improving formulation stability (Figure 1B,C).

To synthesize MoNi, we employed reversible addition fragmentation transfer (RAFT) controlled radical polymerization on account of its simple implementation and excellent control over the synthesis of copolymers. Although RAFT polymerization has many synthetic advantages, it generates polymers with a reactive trithiocarbonate chain transfer agent (CTA) attached at the Z terminus of the polymer following polymerization, denoted here as MoNi-CTA. We removed the CTA moiety from the MoNi-

CTA copolymer to produce the MoNi excipient before utilization in subsequent assays to ensure both chemical stability and biological inertness (**Figure 2**A–D). Absence of the absorbance peak in the size exclusion chromatography (SEC) trace confirms removal of the UV-active Z group (UV$_{max}$/RI$_{max}$ $\approx$ 7 before cleavage and <0.1 after cleavage; Figure 2C). In previous studies, we have demonstrated that MoNi is highly biocompatible and exhibits exceptionally low cytotoxicity compared to typical surfactant molecules; in particular, the MoNi copolymer excipient is synthesized at molecular weights well below the glomerular filtration threshold to ensure that they are rapidly excreted following administration in the body without bioaccumulation.[19] Further, by comparing reported values for the concentration whereby 50% of cells are killed in vitro, a parameter called the LC$_{50}$, MoNi is over 100-fold less cytotoxic than common commercial surfactant excipients such as Polysorbate 80 (PS80) and Pluronic L61 (Figure 2E). Additionally, we attempted to determine MoNi's CMC by performing dynamic light scattering (DLS) analysis on a dilution series of MoNi from 100 to 1 mg mL^{-1} (Figure 2F). In these assays, the MoNi excipient did not form micelles or aggregates within the concentration range assessed. This behavior is unique compared to the common commercial surfactant excipients PS80 and Pluronic L61, which exhibit CMC values well below 1 mg mL^{-1} (Figure 2G). Importantly, most surfactants are toxic in their micellular state, suggesting that the significantly lower cytotoxicity of MoNi may arise from the fact that it does not exhibit self-assembly into micelles at formulation-relevant concentrations.[20]

We then sought to evaluate our hypothesis that MoNi preferentially adsorbs to the air–water interface, thereby precluding mAb adsorption to the interface and improving formulation stability by reducing interface-mediated aggregation events. We first conducted time-resolved surface tension experiments with formulations of the anti-HIV bnAb PGT121, a promising candidate for passive immunization against HIV targeting the well conserved V1/V2 glycan on the gp120 surface glycoprotein that is currently being evaluated clinically.[24,25] We observed that formulations of PGT121 (20 mM acetate buffer, pH $\approx$ 5.0) with MoNi (0.01 wt%) exhibited lower surface tension values compared to PGT121 alone (53 mN m^{-1} and 62 mN m^{-1}, respectively; **Figure 3**A). The lower surface tension observed for the PGT121 formulation comprising MoNi suggests that there are more species packed at the interface in this formulation than in the formulation with only PGT121, indicating that MoNi is preferentially adsorbing to the interface at higher levels. Notably, the surface tension of both a formulation of MoNi only in buffer and a formulation of PGT121 and MoNi were found to be identical (Figure 3A), indicating that the air-water interface is indeed dominated by MoNi at these concentrations. These results suggest that MoNi preferentially adsorbs to the air-water interface, precluding mAb adsorption.
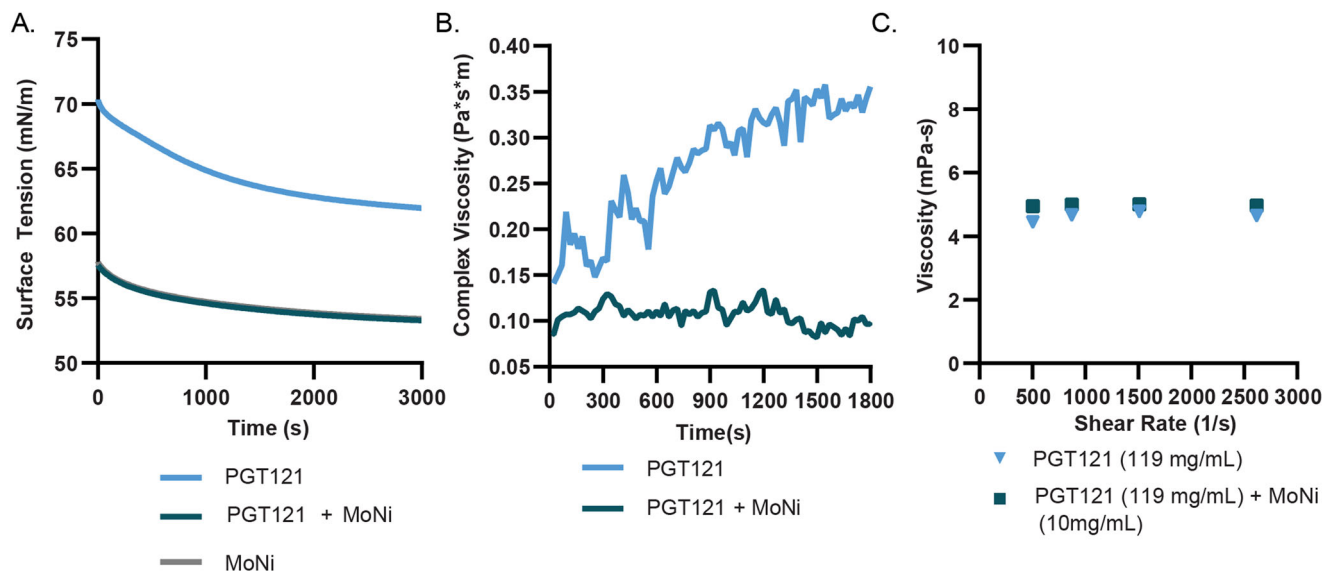
Associative mAb interactions at interfaces leads to the formation of a viscoelastic "skin" and the elasticity of this interface has been shown to correlate strongly with mAb aggregation.[26,27] Therefore, to further characterize the effects of MoNi on the formulation of PGT121, we probed the interfacial viscoelasticity of these formulations via interfacial shear rheology measurements. These measurements revealed that addition of MoNi to PGT121 formulations reduced the interfacial complex viscosity

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Figure 2. Synthesis and characterization of MoNi copolymer excipient. A) Synthetic scheme for the synthesis of poly(acryloylmorpholine-*co*-*N*-isopropylacrylamide) (MoNi). Size exclusion chromatography (SEC) characterization of the B) MoNi-CTA (chain transfer agent) intermediate and C) MoNi demonstrates that the CTA is completely removed in the second step of the synthesis, yielding a chemically stable copolymer. D) ^{1}H-NMR characterization of MoNi. E) Comparison of cytotoxicity of MoNi,[19] polysorbate 80,[21] and pluronic L61.[20] F) DLS characterization of MoNi in solution at various concentrations (5 DLS measurements per sample, mean). G) Comparison of critical micelle concentration (CMC) values for polysorbate 80,[22] and pluronic L61.[23] MoNi did not exhibit micelle formation within the assessed concentration range (100–1 mg mL^{-1}).

by threefold (from ≈0.3 to ≈0.1 Pa s^{-1} m^{-1}; Figure 3B). The high interfacial complex viscosity of the PGT121 formulation without MoNi suggests that protein interactions at the interface are indeed leading to the formation of a gel-like skin at the interface. Consequently, the significant reduction in interfacial complex viscosity upon addition of MoNi indicates that the copolymer excipient reduces interfacial interactions of PGT121, commensurate with the surface tension measurements discussed above

that show that the mAbs are precluded from adsorbing to the interfaces. While associative mAb interactions at the interface are reduced upon addition of MoNi, we also sought to determine whether MoNi interacted with the mAbs in the bulk, which could also potentially lead to conformational changes and subsequent aggregation. Using diffusion ordered spectroscopy, which is a well-established NMR technique that provides direct evaluation of the diffusion behavior of different species in solution, we

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Figure 3. Interfacial interactions of the MoNi polymer excipient in mAb formulations. A) Comparison of PGT121 (0.2 mg mL^{-1}) with and without MoNi (0.1 mg mL^{-1}), and MoNi alone (0.1 mg mL^{-1}), in time resolved surface tension measurements ($n = 3$). PGT121 with MoNi and MoNi alone overlap. B) Interfacial rheology measurements ($n = 3$) of PGT121 (0.2 mg mL^{-1}) with and without MoNi (0.1 mg mL^{-1}). C) High shear rate viscosity measurements collected at steady state by viscometry experiments for high concentration formulations of PGT121 (119 mg mL^{-1}) with and without MoNi (10 mg mL^{-1}).

demonstrated that the PGT121 mAb and MoNi exhibit distinct diffusion behavior (Figure S1, Supporting Information), suggesting that the two species do not interact in the bulk. These results support our hypothesis that MoNi primarily acts to stabilize protein cargo by preventing interfacial aggregation.

With the goal of formulating mAbs at high concentration for SC administration, it was important to verify that formulations with concentrations greater than 100 mg mL^{-1} were easily injectable under clinically relevant injection conditions.[28] We measured the viscosity of high-concentration PGT121 (119 ± 7 mg mL^{-1}) solutions with and without MoNi (10 mg mL^{-1}) at shear rates that are typically achieved during injection through a standard syringe and needle geometry and flow rate. Addition of MoNi at this high concentration did not alter the injection viscosity of the formulation, and both formulations exhibited sufficiently low viscosities to be easily injected (Figure 3C).

2.2. MoNi Polymer Excipient Stabilizes Monoclonal Antibody Formulations in Stressed Aging Assays

The interfacial rheology and surface tension experiments provide strong evidence for preferential adsorption of MoNi to interfaces in formulations with mAbs. To determine whether this behavior also conferred a stability benefit through inhibition of aggregation-nucleating events at the interface, we conducted accelerated aging assays. Initially, we compared a stock PGT121 formulation at 55.5 mg mL^{-1} (20 mM acetate, 9 wt% sucrose, 0.01 wt% PS80, pH ≈ 5.0) with MoNi (1 mg mL^{-1}, 10 mg mL^{-1}) based on prior concentration screens with MoNi in insulin formulations.[19] These antibody formulations were packaged in glass vials and subjected to accelerated aging. The monomeric mAb content in these formulations was monitored over 1 week

with SEC. While formulations containing MoNi at either concentration resulted in greater preservation of monomeric PGT121 over 7 days than in the formulation without MoNi, the sample containing 10 mg mL^{-1} MoNi exhibited the best stability, and thus we moved forward with this excipient concentration for the remainder of this study (Figure S2, Supporting Information). Next, we compared a stock PGT121 formulation at 55.5 mg mL^{-1} (20 mM acetate, 9 wt% sucrose, 0.01 wt% PS80, pH ≈ 5.0) to a formulation concentrated more than twofold at 119 ± 7 mg mL^{-1} (denoted as high concentration). These formulations with and without MoNi (10 mg mL^{-1}) were packaged in glass vials and subjected to accelerated aging as before (constant agitation at 50 °C; **Figure** 4A). The monomeric mAb content in these formulations was monitored over 4 weeks with SEC (Figure 4B). Within 5 days, both the stock and high concentration PGT121 formulations had lost more than 50% of their monomeric mAb content, indicating significant aggregation of the protein. The loss of monomeric PGT121 and formation of aggregates is shown in representative SEC chromatograms (Figure S3, Supporting Information). By day 7, both formulations had macroscopically aggregated, becoming visibly opaque. In contrast, the stock PGT121 formulation comprising MoNi maintained greater than 95% monomeric mAb content through 3 weeks of continuous stressed aging (Figure 4B). While the high concentration sample comprising MoNi exhibited a small initial decrease in monomer mAb content over the first week of stressed aging, likely arising from our concentration process, this formulation maintained near 70% monomer mAb content over the same 3-week period (Figure 4B).

For biopharmaceuticals, preservation of monomeric structure is necessary but not sufficient for maintaining therapeutic activity. Critical to the activity of antibody-based therapeutics is the conservation of structural domains in the antibody's binding epitopes. While the MoNi excipient prevented formation of
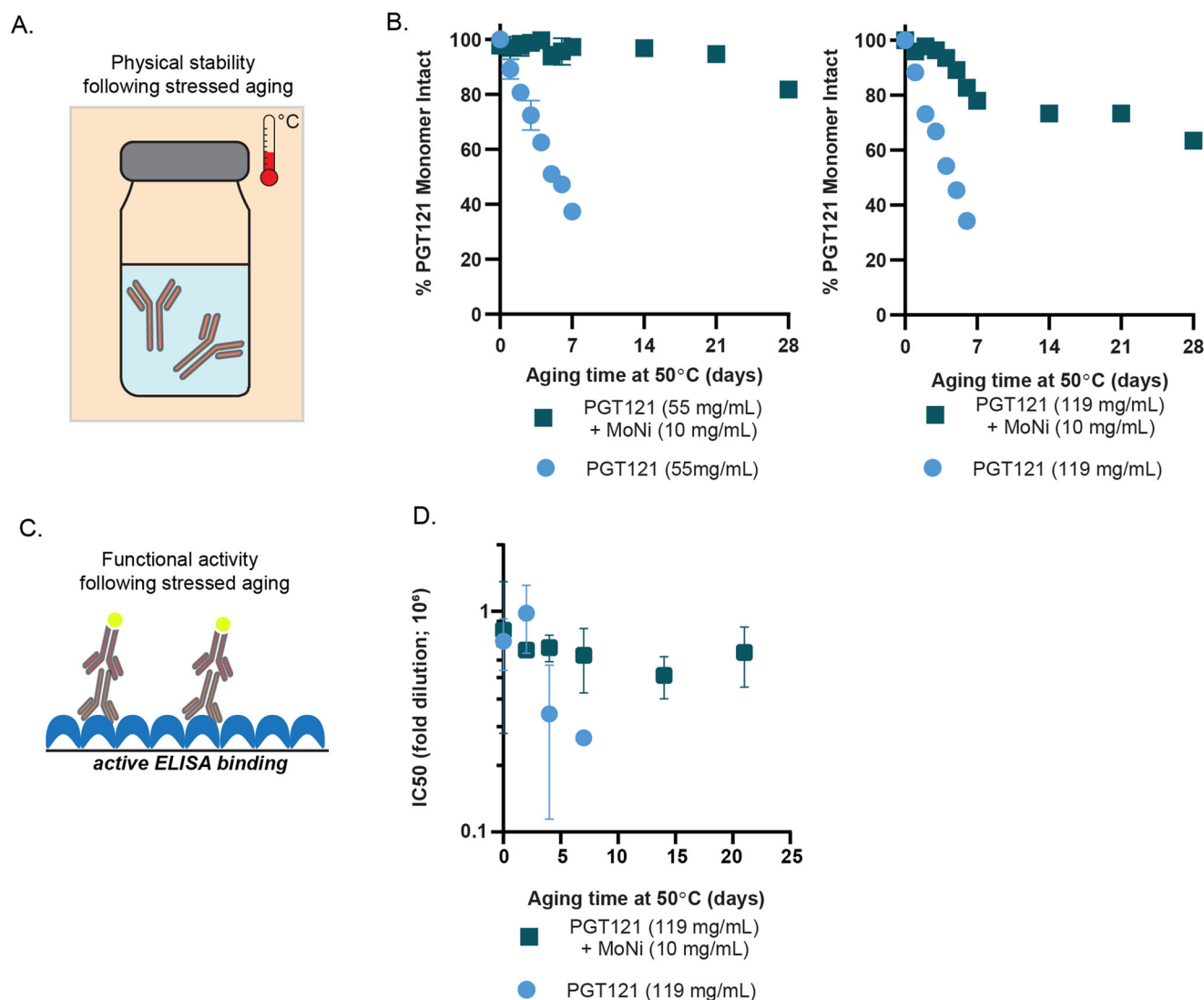
Figure 4. MoNi excipient stabilizes PGT121. A) Schematic of accelerated aging, whereby formulations are packaged in glass vials, agitated, and heated. B) Percent monomeric composition of aged samples determined via SEC. C) Schematic of assaying epitope binding via ELISA. D) Half maximal binding dilutions of aged formulations with and without MoNi. Samples assayed in duplicate.
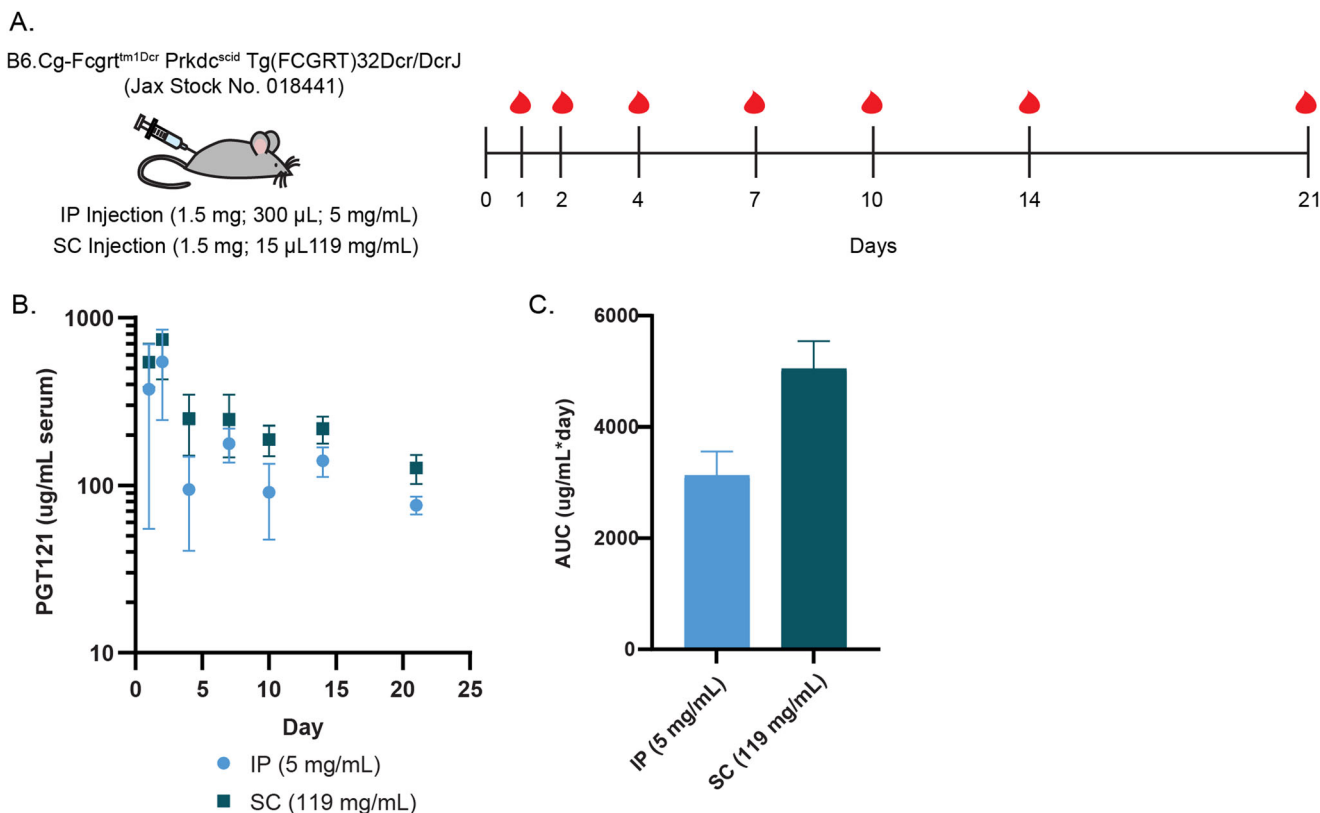
aggregates as determined by SEC, we next sought to determine whether binding epitope activity (as a proxy for therapeutic efficacy) was preserved through continuous stressed aging. We used an enzyme-linked immunosorbent assay (ELISA) to determine whether samples subjected to stressed aging retained binding activity; specifically, we utilized an ELISA with gp120, an envelope trimer of HIV, as its substrate to evaluate Fab binding of PGT121 to its native target. Further, by using a secondary antibody specific for the Fc domain, the assay readout ensures conformational integrity of both Fab and Fc domains of the PGT121 (Figure 4C). We report half maximal binding dilutions (IC_{50}) to compare the prevalence and the functional potency of the high concentration formulations with and without MoNi (10 mg mL^{-1}) subjected to stressed aging. In the absence of MoNi, the PGT121 mAbs rapidly lost functionality, losing more than 65% of their potency after just 5 days of stressed aging (Figure 4E). In contrast, the addition of MoNi significantly enhanced formulation stability and the

PGT121 mAbs retained more than 75% of their original potency through 21 days of continuous stressed aging.

Taken together, these data indicate that the addition of the MoNi excipient to high concentration mAb formulations confers a substantial stability benefit by precluding mAb adsorption to the interfaces, thereby preventing aggregation events and maintaining binding activity under accelerated aging conditions.

2.3. Pharmacokinetic Profile of MoNi-Stabilized High Concentration PGT121 Formulation

To further demonstrate the potential of MoNi as an excipient for mAb formulations, we sought to confirm that it functions as an inactive ingredient by conducting a pharmacokinetic study in rodents. We administered 1.5 mg of PGT121 in transgenic SCID mice with humanized FcRn receptors ($n = 6$/group; B6.Cg-

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Figure 5. Pharmacokinetics of MoNi-stabilized PGT121 A) Experimental scheme, indicating injection dosing and blood collection timeline. B) Serum concentration of PGT121 over time, as determined by ELISA ($n = 6$/group). C) Area under the curve analysis of ELISA data.

Fcgrt[tm1Dcr] Prkdc[scid] Tg[FCGRT]32Dcr/DcrJ; Jackson Labs No. 01 8441) via either SC injection of 15 µL of MoNi-stabilized (1 wt%) high concentration PGT121 (119 mg mL^{-1}) or IP injection of 300 µL of low concentration PGT121 (5 mg mL^{-1}). Serum was collected for 3 weeks post administration to be analyzed for systemic PGT121 concentration via ELISA (**Figure 5**A,B).

Quantification of serum concentration over time as well as total PGT121 exposure (area-under-the-curve) demonstrated comparable pharmacokinetics and bioavailability between the two routes of administration and formulations (Figure 5C). We did not observe a therapeutic difference in terms of serum titer nor bioavailability of PGT121 when administered in a MoNi-stabilized, high concentration formulation. Moreover, no acute weight loss (a common metric for treatment related toxicity) was observed, corroborating previous characterization of MoNi's biocompatibility and tolerability (Figure S4, Supporting Information).[18,19]

Overall, the addition of MoNi to high concentration PGT121 mAb formulations significantly improves their stability without altering pharmacokinetics, potentially enabling the development of clinically relevant SC formulations

3. Conclusion

In this study, we report on the use of MoNi, an amphiphilic copolymer of acryloylmorpholine and *N*-isopropylacrylamide, as a promising excipient to stabilize high concentration mAb for-

mulations. With an increasing market share and over 64 therapeutic mAbs approved by the FDA,[29] biopharmaceuticals are becoming the predominant class of therapeutics. Unfortunately, producing stable biopharmaceutical formulations at relevant concentrations remains a challenge, as increasing formulation concentration increases the likelihood of aggregation. We had previously developed the copolymer excipient MoNi[19] for stabilization of insulin, a small protein therapeutic. We hypothesized that MoNi would confer similar stability benefits to mAb formulations by competitively occupying interfaces, such as the air-water interface, reducing the likelihood of interfacially-mediated aggregation of a well-studied anti-HIV bnAb in formulation. Surface tension and interfacial rheology experiments of the PGT121 mAb formulations containing MoNi indicated that the copolymer excipient preferentially adsorbed to the air-water interface, significantly reducing surface tension, and preventing the formation of a viscoelastic gel-like skin of aggregated mAb at the interface.

Further, both low (55.5 mg mL^{-1}) and high (119 mg mL^{-1}) concentration PGT121 solutions formulated with MoNi (10 mg mL^{-1}) almost completely maintained monomeric mAb content for over 3 weeks when subjected to stressed aging by constant agitation at 50 °C. In contrast, standard mAb formulations completely aggregated in less than a week under these conditions. Moreover, the ELISA binding activity, which is a good proxy for therapeutic efficacy, was preserved over these same timeframes. Indeed, high concentration PGT121 formulations containing MoNi maintained greater than 75% of their binding activity for

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more than 3 weeks of constant stressed aging, whereas standard formulations lost over 65% of their binding activity in only 5 days under the same conditions.

When used as a simple "drop-in" excipient, MoNi does not alter the viscosity of high concentration mAb formulations, as measured by high shear rate viscometry reflective of injection using a standard syringe and needle. Additionally, we demonstrated that the addition of MoNi as an excipient does not alter the pharmacokinetic profile nor the bioavailability of PGT121 in a humanized FcRn mouse model when administered as part of a stabilized high PGT121 concentration formulation injected SC compared to a standard low PGT121 concentration (5 mg mL^{-1}) formulation injected IP.

Together, the facile synthesis of MoNi with a scalable controlled radical polymerization technique, its high degree of biocompatibility and low toxicity,[18,19] and its demonstrated ability to enhance the stability of high concentration biopharmaceutical formulations without altering their pharmacokinetics demonstrate its potential as a simple "drop-in" excipient to improve biopharmaceutical formulations. Enabling stable, high concentration biopharmaceutical formulations can facilitate moving away from IV administration of these drugs in certain applications and more broadly contribute to improved shelf life and a reduction in cold chain dependence.

4. Experimental Section

Materials: Solvents *N,N*-dimethylformamide (DMF; >99.7%; HPLC grade, Alfa Aeser), ethanol (EtOH; >99.5%; Certified ACS, Acros Organics), acetone (>99.9%; Sigma-Aldrich, HPLC Grade), hexanes (>99.9%; Thermo Fisher Scientific, Certified ACS), ether (anhydrous, >99%; Sigma-Aldrich, Certified ACS), and CDCl$_3$ (>99.8%; Acros Organics) were used as received. 4-acryloylmorpholine (MORPH, >97%; Sigma-Aldrich) was filtered with basic alumina before use. *N*-isopropylacrylamide (NIP, >99%; Sigma-Aldrich) was used as received. RAFT CTA 2-cyano-2-propyl dodecyl trithiocarbonate (2-CPDT; >97%; Strem Chemicals) was used as received. Initiator 2,2-azobis(2-methyl-propionitrile) (AIBN; >98%; Sigma-Aldrich) was recrystallized from methanol (>99.9%; Thermo Fisher Scientific, HPLC grade) and dried under vacuum before use. Z group removing agents lauroyl peroxide (LPO; 97%; Sigma-Aldrich) and hydrogen peroxide (H$_2$O$_2$; 30%; Sigma-Aldrich) were used as received. PGT121 mAb was provided by Just-Evotec Biologics, Inc. (Seattle, WA, USA) in collaboration with the Bill and Melinda Gates Foundation. PGT121 was supplied at 55.5 mg mL^{-1} in 20 mM acetate buffer with 9% (w/v) sucrose, 0.01% PS 80, pH 5.0.[24] 9e9 anti-idiotype mAb was synthesized by the Protein Production Facility funded by the Bill and Melinda Gates Foundation, using a plasmid provided by Dr. John Mascola at the Vaccine Research Center (VIRC), a division of the National Institute of Allergy and Infectious Diseases (NIAID) in the US National Institutes of Health (NIH).

Size Exclusion Chromatography Characterization: M_n, M_w, and Đ for MoNi was determined via SEC implementing PEG standards (American Polymer Standards Corporation) after passing through two SEC columns (inner diameter, 7.8 mm; M_w range, 200–600,000 g mol^{-1}; Resolve Mixed Bed Low divinylbenzene (DVB) (Jordi Labs)) in a mobile phase of DMF with 0.1 M LiBr at 35 °C and a flow rate of 1.0 mL min^{-1} (Dionex UltiMate 3000 pump, degasser, and autosampler (Thermo Fisher Scientific)).

NMR Characterization: NMR spectra were recorded in CDCl$_3$ on a Varian 600 MHz and δ values are given in parts per million (ppm).

Synthesis of MoNi: MoNi was synthesized via RAFT, as described previously.[15] Briefly, MORPH (645 mg, 4.57 mmol, 41.5 eq.), NIP (105 mg, 0.93 mmol, 8.5 eq.), 2-CPDT (38 mg, 0.11 mmol, 1 eq.), and AIBN (3.6 mg, 0.02 mmol, 0.2 eq.) were combined and diluted with DMF to a total volume of 2.25 mL (33.3 (w/v) vinyl monomer concentration) in an 8-mL scintillation vial equipped with a PTFE septa. The reaction mix-

ture was sparged with nitrogen gas for 10 min and then heated for 12 h at 65 °C. To remove the Z terminus of the resulting polymer, AIBN (360 mg, 2.2 mmol, 20 eq.) and LPO (88 mg, 0.22 mmol, 2 eq.) were added to the reaction mixture, which was then sparged with nitrogen gas for 10 min and heated for 12 h at 90 °C. Z group removal was confirmed by the ratio of the refractive index to ultraviolet (310 nm) intensity in SEC analysis. Resulting polymers were precipitated three times from ether, dried under vacuum overnight, and characterized. SEC: M_n = 3059 Da, M_w = 3386 Da, and Đ = 1.11.

Dynamic Light Scattering: DLS measurements of MoNi, prepared in Milli-Q water at 1, 5, 25, 50, and 100 mg mL^{-1}, were recorded on a DynaPro Plate Reader II, Wyatt Technology (5 acquisitions per sample, average reported).

Time-Resolved Surface Tension Measurements: Time-resolved surface tension of the air–solution interface was measured with a platinum/iridium Wilhelmy plate connected to an electrobalance (KSV Nima, Finland). The Wilhelmy plate was partially immersed in the aqueous solution in a Petri dish, and the surface tension of the interface was recorded for 50 min from the formation of a fresh interface. Equilibrium surface tension values (t = 50 min) were reported as these values more closely describe the environment in a stored vial prior to agitation. Samples were diluted in a 20 mM acetate buffer without sucrose and surfactant, pH = 5.0, from stock samples to the desired assay concentrations. The experiment was repeated in triplicate.

Interfacial Rheology: Interfacial shear rheology was measured using a Discovery HR-3 rheometer (TA Instruments) with an interfacial geometry comprising a Du Noüy ring made of platinum/iridium wires (CSC Scientific, Fairfax, VA, catalog no. 70 542 000). Before each experiment, the Du Noüy ring was rinsed with ethanol and water and flame treated to remove organic contaminants. The solution chamber consisted of a double-wall Couette flow cell with an internal Teflon cylinder and an external glass beaker. A time sweep was performed at a strain of 1% (within the linear regime) and a frequency of 0.05 Hz (sufficiently low such that the effects due to instrument inertia will not be significant). Interfacial complex shear viscosity was measured for 30 min. Samples were diluted in a 20 mM acetate buffer without sucrose and surfactant, pH = 5.0, from stock samples to the desired assay concentrations. The experiment was repeated in triplicate.

Viscometry: A Rheosense m-VROC viscometer with a low viscosity chip was used to measure the viscosity at high shear rates representative of injection. Samples were measured from low to high shear rates using a Hamilton syringe. Each data point was collected at steady state.

Preparation of High Concentration PGT121 Formulations: PGT121 mAb was concentrated via spin filtration (Corning Spin-X UF 5 kDa MWCO) at 3030 RPM until desired volume recovery. Concentrated formulations were refrigerated for 12 h before usage in accelerated aging studies. Concentrations of PGT121 formulations were determined by either volume recovery, ELISA, Nanodrop (Thermo Scientific) or aqueous SEC-UV (PBS buffer, 300 ppm sodium azide), depending on the assay. For aqueous SEC-UV, PGT121 aliquots were diluted 133X in Milli-Q water and concentration was determined by comparing the area under the curve of the traces obtained from SEC using a Dionex UltiMate 3000 VWD at 280 nm (Thermo Scientific). For nanodrop, the protein concentration was compared to a stock standard at 55.5 mg mL^{-1}. For ELISA, concentrations were interpolated by fitting a four-parameter dose-response curve (variable slope) in GraphPad Prism 9. The high concentration PGT121 formulation is reported as the mean ± standard deviation of the concentration determined by these methods for several preparations of these formulations.

In Vitro Stability Assay: 150 µL of PGT121 solutions (55.5 mg mL^{-1}, 119 mg mL^{-1}) were diluted with 7.5 µL of MoNi in the formulation buffer at 21 mg mL^{-1} to reach a final excipient concentration of 0.1 wt%. These formulations were agitated in glass vials at 200 rpm on an orbital shaker plate in an incubator at 50 °C. 5 µL aliquots were removed every 24 h and stored at 4 °C prior to analysis. Percent monomeric composition and the functional binding activity of aged samples were compared to unaged samples via SEC and ELISA, respectively, as described above.

PGT121 Enzyme-Linked Immunosorbent Assay: The capture antibody, 9e9, was coated at 2 µg mL^{-1} in phosphate buffered saline (PBS) (25 µg

per well) on Corning 96-well high binding flat-bottom half-area microplates (Fisher Scientific) and incubated at 4 °C overnight. Blocking buffer used was 2% non-fat dry milk in PBS, assay buffer used was 2% bovine serum albumin in PBS, and wash buffer was PBS-T (0.05% Tween 20). Plates were washed twice and blocked with 125 µL of blocking buffer and incubated for 1 h at room temperature. Sample dilutions and PGT121 standards were prepared in assay buffer. After blocking, assay buffer was removed from the plate before adding 50 µL of each sample and incubating for 1 h at room temperature. Plates were washed five times before adding 50 µL per well of the secondary antibody, Peroxidase AffiniPure F(ab')$_2$ Fragment Goat Anti-Human IgG, Fcγ fragment specific (Jackson Immunoresearch, 109-036-008, RRID: AB╲_2 337 591), diluted 1:5000 in assay buffer. After incubating for 1 h at room temperature, plates were washed 10 times and then developed by adding 50 µL of TMB ELISA Substrate (High Sensitivity) (Abcam, ab171523). Development was stopped after 2.5 min by adding 1 N hydrochloric acid. PGT121 concentration was measured by absorbance intensity at 450 nm using a Synergy H1 Hybrid Multi-Mode Plate Reader (BioTek). Each plate contained a 15-point standard curve assayed in duplicate which was used to interpolate PGT121 concentrations by fitting a four-parameter dose-response curve (variable slope) in GraphPad Prism 9. Stressed aging samples were diluted 1:10 000 and then serially diluted by 4x to create a 7-point curve assayed in duplicate for each sample. IC$_{50}$ values were determined by fitting a four-parameter dose-response curve (variable slope) in GraphPad Prism 9. Mean value and standard deviation reported.

In Vivo Pharmacokinetic Study: Animal studies were conducted in accordance with the guidelines for care and use of laboratory animals under protocols approved by the Stanford Institutional Animal Care and Use Committee (IACUC). 8 week old female B6.Cg-Fcgrt^{tm1Dcr} Prkdc^{scid} Tg(FCGRT)32Dcr/DcrJ (The Jackson Laboratory, Stock No. 01 8441) mice were administered PGT121 antibody (1.5 mg per mouse) via IP or SC injection under brief isoflurane anesthesia ($n = 6$/group). This mouse strain was chosen because it is specifically engineered to allow for the study of the human IgG antibody pharmacokinetics.[30,31] The polymer-stabilized high concentration SC formulation was prepared with 102 mg mL^{-1} PGT121 (as determined by ELISA) and 1 wt% MoNi (15 µL injection volume), and the low concentration IP formulation was prepared at 5 mg mL^{-1} (300 µL injection volume) in the same buffer in which the antibody was provided. Blood samples were collected at 24 h, 48 h, and then days 4, 7, 10, 14, and 21 post-injection for analysis of PGT121 serum concentration via ELISA.

Statistical Analysis: All results are expressed as mean ± standard deviation (SD) unless specified otherwise and analyzed using Graphpad Prism 9.1 (GraphPad Software Inc, USA). Data processing, data presentation and sample size and/or technical replicates are fully described in pertinent methods sections.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

Conflict of Interest

J.H.K, C.M.K., J.L.M., and E.A.A. are listed as inventors on a patent application related to this work. The remaining authors declare no competing financial interests.

Data Availability Statement

All data supporting the results in this study are available within the article and its Supplementary Information. The broad range of raw datasets acquired and analyzed (or any subsets thereof), which would require contextual metadata for reuse, are available from the corresponding author upon reasonable request.

Keywords

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