Identifying the Risk of Infusion Reactions

In vitro assessment of complement activation, cytokine expression and platelet aggregation

Assays designed to assess the potential for infusion reactions

NCL Nanotechnology Characterization Laboratory

Nanomedicines and Infusion Reactions

Many nanomedicines are designed to be administered systemically. However, severe reactions to the infusion of these therapies often delays or halts their clinical translation. These reactions can manifest as fevers, chills, rigors, rashes, chest or back pain, or difficulty breathing, and, in rare instances, they can be fatal.

Although not unique to nanomedicines, infusion reactions pose a significant hurdle for nanomedicines because of the complex nature of nanoformulations and the nuanced approval process for these products. Identifying the risk of infusion reactions early in the drug development process can help mitigate potential safety concerns once the product reaches clinical trials, saving developers both time and money, and saving patients from potentially dangerous complications.

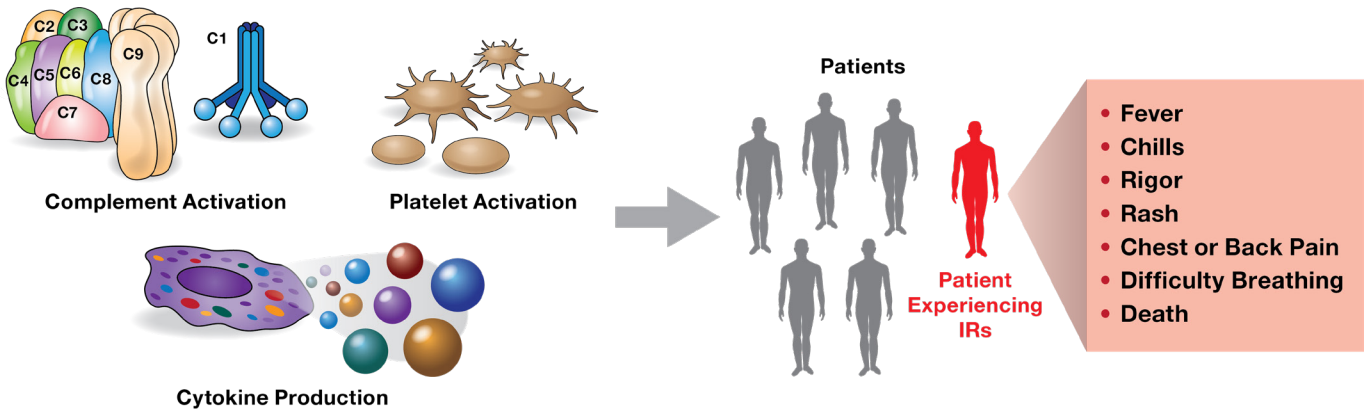
Three assays, when analyzed together, can be indicative of infusion reactions: complement activation, platelet aggregation, and inflammatory cytokine expression assays. Each assay addresses one of the mechanisms currently known to trigger infusion reactions, and together, they offer a useful and comprehensive package that can help investigators understand the potential risk.

For more background on nanoparticle-mediated infusion reactions, read Szebeni, et al, Nature Nanotechnology. 2018, 13(12), 1100-1108.

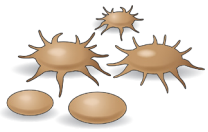
NCL-05: The service includes three in vitro assays designed to identify possible infusion reactions:

1) **Platelet Aggregation:** In vitro analysis of a nanoparticle's ability to induce platelet aggregation and its effect on collagen-induced platelet aggregation.
2) **Complement Activation:** In vitro analysis of a nanoparticle's ability to induce the classical, alternative and common pathways of complement activation, and formation of the terminal complex.
3) **Cytokines:** In vitro analysis of a nanoparticle's ability to induce secretion of pro-inflammatory cytokines in human peripheral blood mononuclear cells. Assay can be customized to include biomarkers relevant to the test formulation.

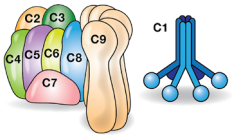
All tests utilize freshly drawn human blood, collected in accordance with all appropriate laws and regulations.



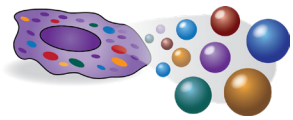
How The Assays Work



Platelet Aggregation. Platelet rich plasma (PRP) and platelet poor plasma (PPP) are prepared from freshly drawn human blood of three healthy individual donors. PRP is incubated with the ChronoLum reagent and test samples, and sample turbidity is measured using the ChronoLog aggregometer. PPP is used as the background control.



Complement Activation. Plasma is prepared from freshly drawn human blood of three healthy individual donors. Plasma is incubated with test samples and veronal buffer for 30 min at 37°C. Following incubation, the samples are analyzed for the presence of the C3a, C4a, and C5a anaphylatoxins, sC5b-9 terminal complex, and Bb component of complement using a commercial custom multiplex ELISA kit (Quidel, San Diego, CA).



Cytokines. Peripheral blood mononuclear cells (PBMC) are prepared from freshly drawn human blood of three healthy individual donors. PBMC are incubated with test samples at 37°C. Incubation time is customized to the given test sample and selected cytokine biomarkers. Following incubation, the samples are centrifuged and supernatants are analyzed for the presence of cytokines and interferons using commercial, custom multiplex ELISA kits (Quansys, Logan, UT). The default cytokine panel uses either 15-plex (IFNγ, IL1-α, IL-1β, TNF-α, IL-6, IL-8, IL-10, IL-12p70, IL-21, IP-10, MCP-1, MCP-2, MIP-1α, MIP-1β, RANTES) or 14-plex (IFNα, IFNβ, IFN-λ, IFN-ω, IL-2, IL-4, IL-5, IL-7, IL-13, IL-15, IL-17, IL-22, IL-23, IL-27) plates.

For more information on the above referenced protocols, please see https://ncl.cancer.gov/resources/assay-cascade-protocols

Importantly, these assays are not 100% predictive—like most in vitro assays. The data are intended to offer a "likelihood" of infusion reactions.

Frequently Asked Questions

Q: Who will benefit from this service?

A: Any developer with a formulation intended for infusion will benefit. Ideally, these assays will be conducted at the preclinical stage of development, thus, minimizing the impact on late-stage development and mitigating potential risks to patients.

Q: How many samples can I submit?

A: The service has been costed for analysis of one sample at up to five different concentrations. In select cases, five samples can be tested at a single concentration. Contact us to learn more.

Q: Are the assays customizable?

A: There is some flexibility in the types of cytokines that can be offered, but not the overall number of cytokines. Please inquire about availability for your specific needs.

Q: What are the requirements?

A: Developers will be expected to supply sufficient amounts of their formulation, along with proper storage, resuspension (if applicable), and handling procedures. Amounts are determined based on the intended dose of the formulation. Contact us for calculation of your material amounts.

Q: When can I expect results/How are results reported?

A: The studies are expected to be completed within one month of sample receipt. A written report will be provided detailing the methods, results and analysis of the data. NCL staff are available for discussion of the data as needed.

Q: Can I submit non-nanomaterial formulations for testing?

A: Yes. While NCL's primary research and testing has been in the field of nanotechnology, these assays can be applied to non-nanomaterial formulations.

Q: How do I access the service?

A: Interested parties simply contact the NCL to complete the necessary paperwork and arrange for payment.

For questions or more information, please contact the NCL at **ncl@mail.nih.gov**

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