Targeting the cGAS-STING Pathway Using a Homogenous, HTS Compatible Transcreener® cGAS Assay

Meera Kumar, Justin Brink, and Robert G. Lowery
BellBrook Labs, Madison, WI, USA

Overview

Cyclic GMP-AMP synthase (cGAS) is a recently discovered enzyme that acts as a foreign DNA sensor to elicit an immune response to pathogens via activation of the STING (stimulator of interferon genes) receptor. Shortly after its discovery in 2013, aberrant activation of cGAS by self-DNA was shown to underlie debilitating and sometimes fatal autoimmune diseases, such as systemic lupus erythematosus (SLE) and Aicardi-Goutières Syndrome (AGS). Knockout studies in animal models have clearly indicated that inhibiting cGAS is a promising approach for therapeutic intervention. However, there are no HTS-compatible assay methods for measuring cGAS enzyme activity or for monitoring cGAMP in cell and tissue samples. To enable HTS efforts targeting cGAS, we developed Transcreener-based assays for cGAS detection, i.e., homogenous, competitive immunoassays with fluorescence polarization (FP) and time resolved Förster resonance energy transfer (TR-FRET) signals. The key assay reagents are antibodies homogenous, competitive immunoassays with fluorescence polarization (FP) and time resolved Förster resonance energy transfer (TR-FRET) signals. The key assay reagents are antibodies.

The cGAS-cGAMP-STING Pathway Activates the Immune System in Response to Cytosolic DNA

Figure 1. IFN-driven immune responses triggered by cGAS/STING are critical for protection against microbial pathogens and for tumor cell-specific T cell responses in cancer, but activation by self-nucleic acids can contribute to autoimmune diseases.

Screening and Triaging of Non-Stoichiometric Inhibitors

Figure 4. A. HTS Workflow: Primary screen and follow up assays used to triage undesirable compounds and select cGAS inhibitors for advancement into medicinal chemistry SAR. B. Scatter plot from a 1600 compound (10 plates) from a 100K screen: compounds at 20 µM (positive controls lacked dsDNA (required for cGAS activation); Z = 0.59, Z* = 0.63. C, D. Confirmation of a screening hit using FP assay (C) and TR-FRET assay (D) for dose response. Average IC₅₀’s were 12.8 µM and 22.7 µM, respectively. E, F, G. Identification of non-stoichiometric inhibitors (NS) using inhibitor titration (E, F) and detergent disaggregation (G, H). Excess enzyme titrates out NSs resulting in a significant increase in IC₅₀ (E.3-5 times). (F, H) Non-ionic detergent can disperse aggregates, resulting in decreased IC₅₀.

Characterizing Mode of Inhibition

Figure 5. Characterization of Inhibitor MOA During Hit-to-Lead Using Substrate Competition Assays. A, B. Identification of a cGAS competitive inhibitor. C, D. Identification of an inhibitor that competes with both ATP and OMP. The Transcreener cGAS FP assay was used for all assays.

Measuring Inhibitor Residence Time

Figure 6. A. Schematic of jump dilution method used to measure inhibitor residence time. B. Monitoring recovery of activity for a series of cell-permeable inhibitors following jump dilution. Activity recovers as the E1 complex dissociates, allowing calculation of koff rates.

Conclusions

- Competitive immunomodulls for cGAMP with FP and TR-FRET readouts were validated for detection of cGAS enzyme activity and small molecule screening.
- The assays served as a critical tool in a cGAS HTS campaign, including screening, hit confirmation and triaging of non-stoichiometric inhibitors.
- The Transcreener cGAS FP assay was used to sort analogs based on mechanism of inhibition during hit-to-lead, which provided valuable information for driving SAR.
- The ability to run the assay in continuous mode allowed measurement of inhibitor residence time in an automated format, allowing incorporation of binding kinetics into SAR.

Mix and Read HTS Enzymatic Assays for cGAS Based on Immunodetection of cGAMP

Figure 2. Transcreener cGAS Assay principle: Enzymatically generated cGAMP displaces a fluorescent tracer from mAb causing a decrease in its polarization or TR-FRET.

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Assay Validation

Figure 3. A. Specifity of mAb: Measuring cGAS initial activity requires detection of cGAMP in an excess of ATP and GTP. B. Detection of purified, full-length human cGAS with FP Assay. cGAS enzyme reactions contained 100 µM ATP and GTP, 62.5 nM 45 bp ISD DNA, 60 min reactions. N- and C-terminal His-tagged cGAS was produced at BBL; 6xHis-cGAS was used at 10 µM final concentration. Wells contained all cGAS enzyme reaction components except the cGAS enzyme.

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