Targeting cGAS for Type 1 Interferon-Driven Autoimmune Diseases

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Overview

Detection of foreign nucleic acids is an important first line of defense in the immune response to microbial pathogens. However, aberrant induction of type I interferons (IFN) by self-nucleic acids causes debilitating autoimmune diseases such as Aicardi–Goutieres Syndrome (AGS), systemic lupus erythematosus (SLE) and Sjogren's syndrome. A number of recent studies have clearly established that a key molecular trigger for nucleic acid-driven type I IFN induction is production of the unique cyclic dinucleotide, cyclic GAMP (cGAMP), by the cytosolic DNA sensor, cyclic GAMP synthase (cGAS).

To enable screening for cGAS inhibitors, we developed a cGAS enzymatic assay with fluorescence polarization (FP) and time-resolved Forster resonance energy transfer (TR-FRET) readouts based on our Transcreener® HTS platform. We used the FP assay to screen 100,000 compounds with full length human cGAS, resulting in the identification of four novel chemotypes. Following confirmation of hits and removal of compounds with structurally evident reactivity or metabolic liabilities, we triaged non-stoichiometric inhibitors, aggregators, DNA intercalators and redox-active compounds using a battery of established assays. Initial studies revealed two promising chemotypes (BBL40783 and BBL50101) with favorable structural, physicochemical and ADME/PK properties that function via distinct mechanisms. BBL40783 exhibited good concordance between biochemical IC_{50} and K_d determined by surface plasmon resonance (1.26 µM, 2.4 µM, respectively). BBL50101 and related analogs did not bind appreciably in SPR but were found to stabilize cGAS in thermal shift assays in the presence of ATP, GTP and dsDNA, suggesting that this chemotype may bind specifically to dimerized cGAS. We used SAR-driven medicinal chemistry with both chemotypes to increase the potency into the nanomolar range. We obtained a high-resolution crystal structure of a BBL40783 analog in complex with cGAS and demonstrated cGAS-specific cellular activity with the same compound. These efforts establish a strong foundation for development of first-in-class lead molecules targeting cGAS for autoimmune disease.

Aberrant Activation of cGAS by Self-DNA Causes Autoimmune Disease

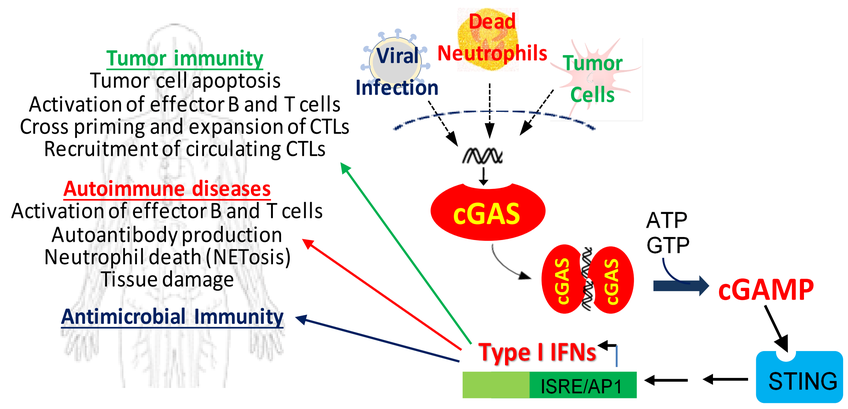

Figure 1. cGAS is a high affinity sensor for cytoplasmic DNA. Binding of dsDNA to the inactive cGAS monomer induces formation of the catalytically active dimer, triggering an innate immune response via transcriptional activation of type I IFN expression. Though critical for anti-microbial and anti-tumor immunity, sustained activation of cGAS causes autoimmune disease.

A Homogenous Enzymatic Assay for cGAS to Enable HTS

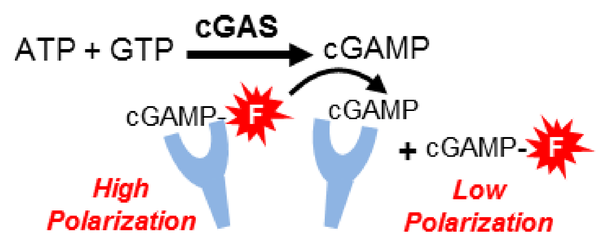

Figure 2. Transcreener cGAS Assay principle: In the competitive fluorescence polarization (FP) immunoassay for cGAMP, enzymatically generated cGAMP displaces a fluorescent tracer from mAb causing a decrease in its polarization.

Development and Validation of a Transcreener cGAS Assay

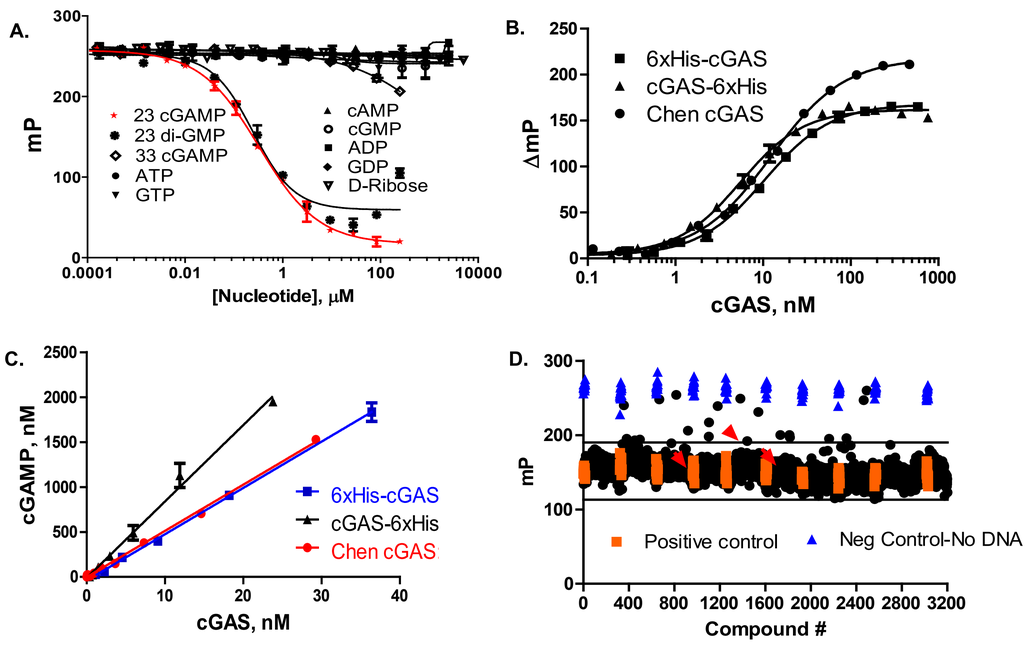

Figure 3. A. Specificity of mAb: Competition binding curves show outstanding selectivity for cGAMP vs. cGAS substrates, ATP and GTP, as well as related molecules. **B. Detection of purified, full-length human cGAS.** cGAS enzyme reactions contained 100 µM ATP and GTP, 62.5 nM 45 bp ISD DNA, 60 min reactions. N- and C-terminal His-tagged cGAS was produced at BBL; 6xHis-cGAS was also generously supplied by Z. Chen (UTSW Medical Center). **C. Linear response**: Polarization data from A. was converted to cGAMP using a standard curve. Rates were 1.2, 0.61 and 0.86 min-1 respectively for 6xHis-cGAS, cGAS-6xHis and Chen cGAS. **D. Scatter plot from 3,200 compounds (10 plates) from a 100K screen**: cGAS reactions essentially as described for B., with 30nM 6xHis-cGAS; compounds at 20 µM; negative controls lacked dsDNA (required for cGAS activation); Z = 0.59, Z' = 0.63.

Discovery and Validation of Novel cGAS Inhibitor Chemotypes

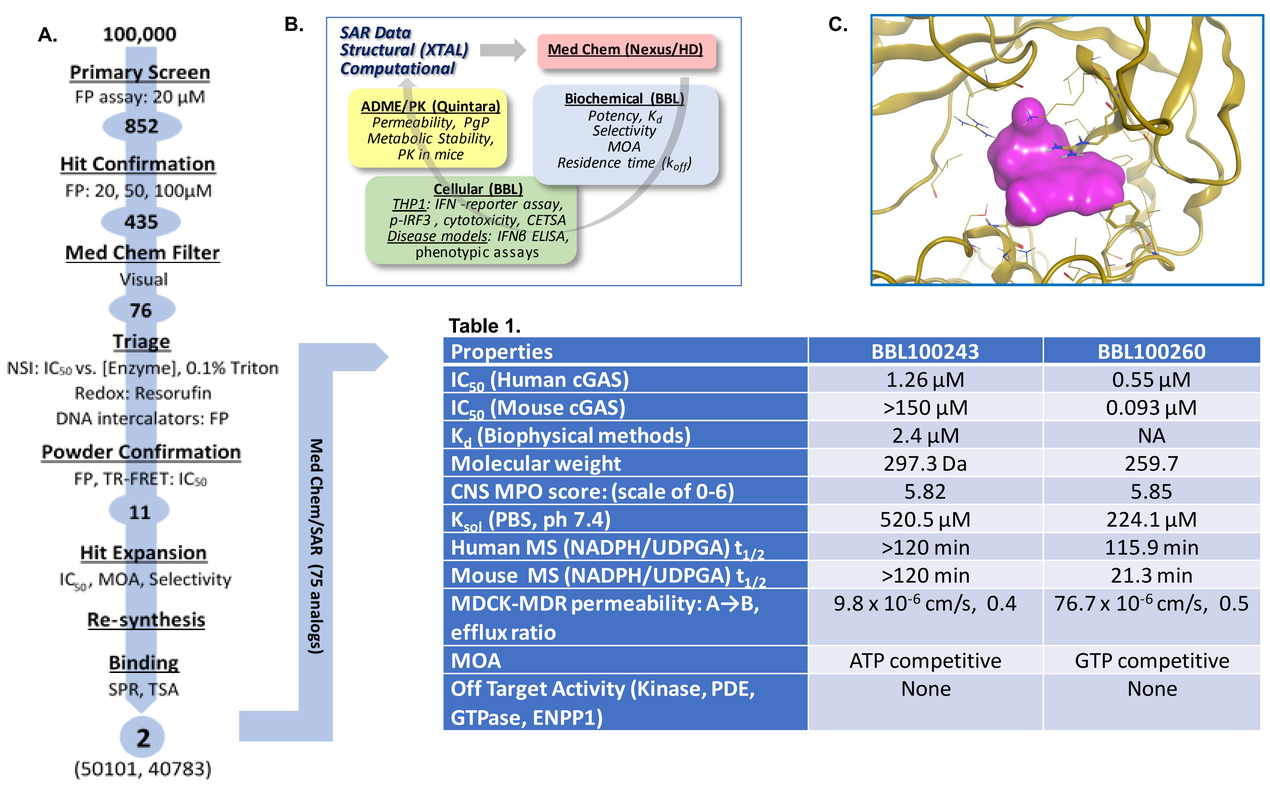

Table 1.

Properties	BBL100243	BBL100260
IC_{50} (Human cGAS)	1.26 µM	0.55 µM
IC_{50} (Mouse cGAS)	>150 µM	0.093 µM
K_d (Biophysical methods)	2.4 µM	NA
Molecular weight	297.3 Da	259.7
CNS MPO score: (scale of 0-6)	5.82	5.85
K_{sol} (PBS, ph 7.4)	520.5 µM	224.1 µM
Human MS (NADPH/UDPGA) $t_{1/2}$	>120 min	115.9 min
Mouse MS (NADPH/UDPGA) $t_{1/2}$	>120 min	21.3 min
MDCK-MDR permeability: A→B, efflux ratio	9.8 x 10^{-6} cm/s, 0.4	76.7 x 10^{-6} cm/s, 0.5
MOA	ATP competitive	GTP competitive
Off Target Activity (Kinase, PDE, GTPase, ENPP1)	None	None

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. NSI- non-stoichiometric inhibition; MOA – mechanism of action; SPR – surface plasmon resonance; TSA – thermal shift assay. **B. Development of cGAS lead molecules, key partners:** Nexus Discovery Advisors, Frederick, MD, Quintara Discovery, Hayward, CA; HD Bio, Shanghai, China; XTAL Biostructures, Natick, MA. **C. Cocrystal structure:** BBL-100243 bound in active site of human cGAS; from X-ray structure of the cocrystal. **Table 1: Key properties of current lead compounds developed from hits 40783 and 50101.** IC_{50} values are means from 2-5 separate determinations ± SD; values for the original hits (40783, 50101) are in parentheses. CNS MPO: central nervous system multiparameter optimization; MS: microsomal; MDCK-MDR: Madin-Darby Canine Kidney cells-Multi Drug Resistance pump; PDE: phosphodiesterase; ENPP1 – ectonucleosidase.

Specific Inhibition of cGAS in Human Monocytes

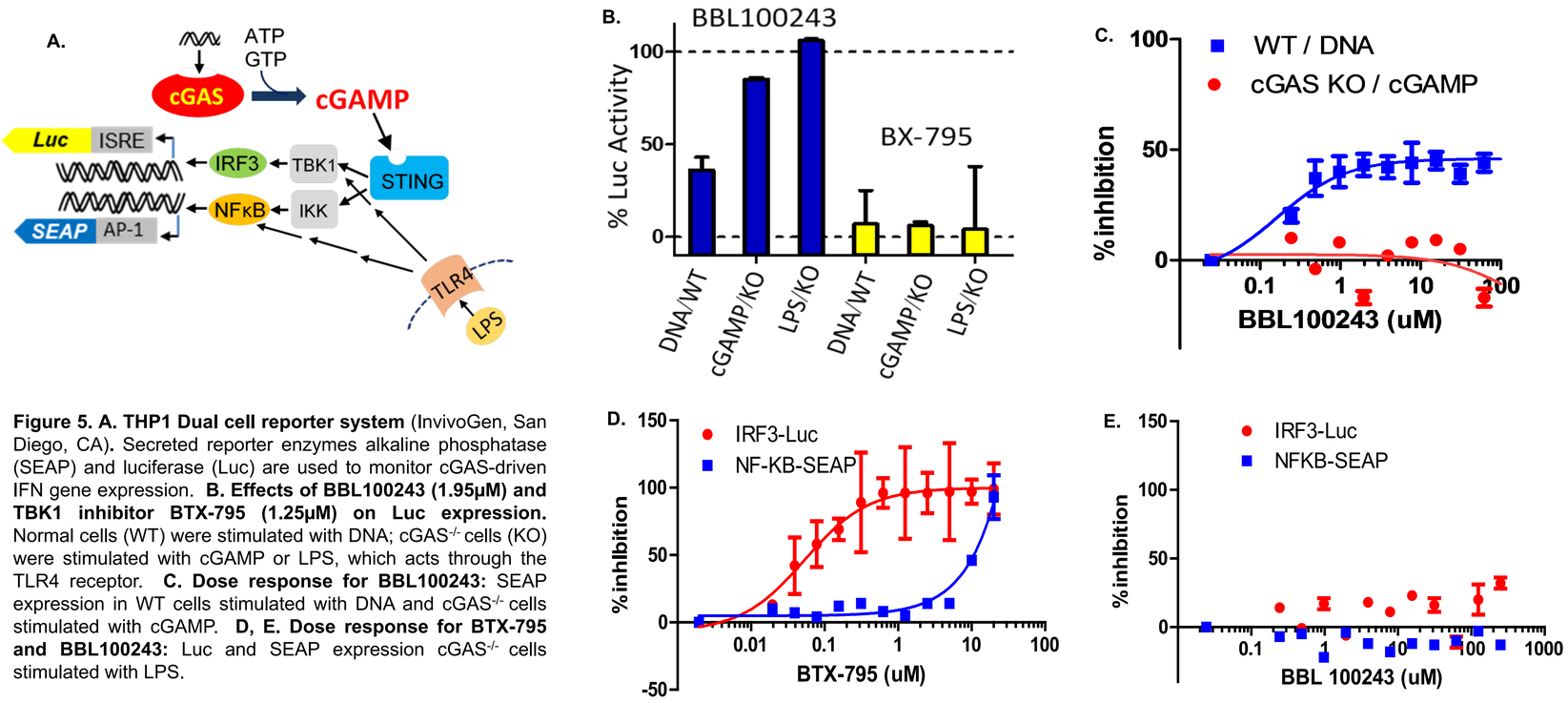

Figure 5. A. THP1 Dual cell reporter system (InvivoGen, San Diego, CA)**.** Secreted reporter enzymes alkaline phosphatase (SEAP) and luciferase (Luc) are used to monitor cGAS-driven IFN gene expression. **B. Effects of BBL100243 (1.95µM) and TBK1 inhibitor BTX-795 (1.25µM) on Luc expression.** Normal cells (WT) were stimulated with DNA; $cGAS^{-/-}$ cells (KO) were stimulated with cGAMP or LPS, which acts through the TLR4 receptor. **C. Dose response for BBL100243:** SEAP expression in WT cells stimulated with DNA and $cGAS^{-/-}$ cells stimulated with cGAMP. **D, E. Dose response for BTX-795 and BBL100243:** Luc and SEAP expression $cGAS^{-/-}$ cells stimulated with LPS.

Conclusions

- ***Competitive immunoassays for cGAMP with FP and TR-FRET readouts were developed to enable quantitative detection of cGAS enzymatic activity in a homogenous format.***
- ***An 100K HTS campaign was used to discover four novel cGAS chemotypes, two of which have been advanced into hit-to-lead.***
- ***The current lead molecules, BBL100243 and BBL100260 have good chemical tractability, no Lipinski violations, CNS MPO scores greater than 5 and favorable ADME/PK properties.***
- ***BBL100243 has been co-crystallized with human cGAS to enable a structure-driven design approach for optimization. It binds in the donor pocket, where ATP is initially bound, and is stabilized by pi stacking interactions with conserved Tyr436.***
- ***BBL100243 inhibited cGAS-driven gene expression in human monocytes at low micromolar concentrations, and had greatly diminished effect on cGAS KO cells stimulated with cGAMP or LPS.***

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