Selective Targeting of Protein Interactions Mediated by Epigenetic Effector Domains

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Seminar at the Universität Duisburg-Essen
Essen, Germany September 5, 2013
Exploring New Target Areas

Divide each protein family into subfamilies, and generate at least one chemical probe for each subfamily with:

- $K_d < 100 \text{nM}$
- $>30$-fold selectivity versus other subfamilies
- Demonstration of “on-target” effect in cells at $\leq 1 \text{uM}$

Probe Criteria

Divide each protein family into subfamilies, and generate at least one chemical probe for each subfamily with:

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Chemical Probe Consortium

- Too large an undertaking and too high risk for individual Pharma companies
- Large global academic community, but lack of quality chemical tools in the public domain
- **Open Access Chemical Probes Consortium** combines expertise in academia and Pharma, and pools resources to effectively evaluate the field

A Large Private Public Partnership

- GlaxoSmithKline
- Pfizer
- Eli Lilly
- Novartis
- Abbvie
- Takeda
- Boehringer
- J&J
- Oxford: SGC Chemistry Department
- NIH Chemical Genomics Centre, Bethesda & Many Academic Collaborators
- Toronto SGC UNC Center for Integrative Chemical Biology and Drug Discovery
- 61 domains in Human
- ~120 residue Kac interaction module
- Clinical POC targeting Kac regulation (HDACs)
- Linked to many diseases
- Druggable pocket

**BRD2: 2DVQ**

*Cell, March 30, 2012*
Identifying High Affinity Substrates

Peptide library screen using SPR

Histone peptide ➔

Targets

Peptide array screens using dot blots

- Affinities can be determined by SPR but not using dot blots
- Many BRDs show no interaction with Histone marks
- 36 BRDs screened against all possible histone Kac sites and combinations of marks.
- 485 new target sequences identified
- BRDs recognize multiple marks

Cell, March 30, 2012
# Bromodomain Binding Assays

<table>
<thead>
<tr>
<th>Assay</th>
<th>Potent Output</th>
<th>Sensitivity</th>
<th>Throughput</th>
<th>Ligand needed</th>
<th>Drawbacks</th>
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</thead>
<tbody>
<tr>
<td>Alpha-Screen</td>
<td>Low IC50</td>
<td>High</td>
<td>High</td>
<td>Yes</td>
<td>High false positive rate</td>
</tr>
<tr>
<td>Tm Shift</td>
<td>High Δ°C</td>
<td>Medium</td>
<td>High</td>
<td>No</td>
<td>Indirect</td>
</tr>
<tr>
<td>Octet BLI</td>
<td>Low Kd</td>
<td>Medium</td>
<td>Medium</td>
<td>No</td>
<td>Biotinylated protein</td>
</tr>
<tr>
<td>Micro ITC</td>
<td>Low Kd</td>
<td>Low</td>
<td>Low</td>
<td>No</td>
<td>Protein consumption</td>
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</tbody>
</table>
Benzo(thieno)diazepines as selective BET inhibitors

GSK WO 2009/54844
(phenotypic screen)

(+)-JQ1
I-BET
MS417
BzT-7

WO 2012075456 A1
Constellation

WO 2013030150 A1
Bayer
Benzo(thieno)diazepines as selective BET inhibitors

Nature Drug Discovery, Sep 2013

GSK 525762
OTX015  Oncoethix
RVX-208  Resverlogix
Basic understanding of transcription

- IFN-induced recruitment of P-TEFb is under control of pausing complex NELF/DSIF.
- BRD4 is associated with small set of exceptionally large super-enhancers associated with genes that feature key oncogenic drivers.
- BET mediates RNA polymerase II (Pol II) S2 phosphorylation.
- RNA polymerase II stalling promotes nucleosome occlusion and pTEFb recruitment to drive immortalization by Epstein-Barr virus.
- BRD4 is required for DNA damage response: Loss of BRD4 results in relaxed chromatin structure, rapid cell-cycle checkpoint recovery and enhanced survival after irradiation.
**JQ1 probe Impact (Cancer)**

**Cancer Biology:**

- **BRD4/NUT** driver tumours are sensitive to JQ1 resulting in terminal differentiation of cancer cells.
- Brd4 as a promising therapeutic strategy in **AML**
- BET bromodomain proteins regulate c-Myc expression in **myeloma**.
- Down regulation of c-Myc and IL7R in B-cell acute lymphoblastic leukaemia (B-ALL)
- BET inhibition suppresses **FOSL1** expression in lung cancer
- Suppression of key oncogenic drivers (c-Myc, p21(CIP1/WAF1), hTERT, Bcl-2, and Bcl-xL) in **glioblastoma**
- BET inhibition increases **sensitivity of standard therapy** (Rituximab resistance in lymphoma and dexamethasone in ALL)

Cell. 2011 Sep 16;146(6):904-17.
JQ1 probe Impact (Inflammation)

Inflammation and Viral Infection:

- BET inhibition reactivate HIV from latency via a Tat-independent mechanism
- JQ1 impairs mouse macrophage inflammatory response
- Enhanced migration, proliferation, and IL-6 release observed in LFs from Idiopathic pulmonary fibrosis patients are attenuated by JQ.
- BRD4 is essential for human papillomavirus type 16 DNA and polyomavirus DNA replication
- BET inhibition potently suppresses cardiomyocyte hypertrophy in vitro and pathologic cardiac remodeling in vivo

Phenylephrine (PE) induced cellular hypertrophy

Cell. 2013 Aug 1;154(3):569-82.

Haldar lab/Cleveland
Consequences of BET inhibition in vivo
Consequences of BET inhibition: Spermatogenesis (BRDT)

Ad Spermatogonium
Ap Spermatogonium
Type B Spermatogonium
Primary/Secondary Spermatocyte

Basal lamina
Sertoli Cell
Mature spermatid
Spermatid
Spermatocyte
Spermatogonia
BTB
Lumen
Consequences of BRDT Inhibition

- Genetic studies of BRDT in mice have demonstrated that deletion of the BRDT(1) is sufficient to confer sterility.
- JQ1 reduced seminiferous tubule area, testis size, and spermatozoa number and motility without affecting serum hormone levels.
- GWAS linked SNPs in BRDT to male infertility.
- JQ1 crosses BTB and accumulates in testis ($\text{AUC}_{\text{testis}}/\text{AUC}_{\text{plasma}} = 259\%$) with a rapid ($T_{\text{max}} = 0.25$ hr) and pronounced exposure ($C_{\text{max}} = 34 \, \mu g/mL$)
- 50 mg/kg of JQ1/daily showed 75% reduction of testis volume after 3 weeks and 54% reduction after 6 weeks

Cell August, 2012
Consequences of BRDT Inhibition
Recovery After BRDT Inhibition

**Low-Dose Regimen**

<table>
<thead>
<tr>
<th>Month</th>
<th>Dose</th>
<th>Frequency</th>
<th>Pups per Litter</th>
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<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>QD</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>75</td>
<td>QD</td>
<td>10</td>
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<tr>
<td>3</td>
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<td>6</td>
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<tr>
<td>4</td>
<td></td>
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<td>4</td>
</tr>
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<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>6</td>
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<td>0</td>
</tr>
<tr>
<td>7</td>
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**High-Dose Regimen**

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<tr>
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</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td>2</td>
</tr>
</tbody>
</table>

**Control**

- Recovery 4 months after JQ1 QD (40x)

**(+)-JQ1**

- Recovery 4 months after JQ1 QD (40x)
Testicle size 'link to father role'

10 September 2013 Last updated at 01:28

A link between the size of a father's testicles and how active he is in bringing up his children has been suggested by scientists.

Researchers at Emory University, US, said those with smaller testicles were more likely to be involved with nappy changing, feeding and bath time.

They also found differences in brain scans of fathers looking at images of their child, linked to testicle size.

But other factors, such as cultural expectations, also played a role.

Levels of promiscuity and testicle size are strongly linked in animals, those with the largest pair tending to mate with more partners.

The researchers were investigating an evolutionary theory about trade-offs between investing time and effort in mating or putting that energy into raising children. The idea being that larger testicles would suggest greater commitment to creating more children over raising them.
Clinical BET Inhibitors (I)
RVX–208 is a Clinical BET Inhibitor Specific for the Second BRDs

- Currently in phase IIb for treatment of atherosclerosis and trial in AD listed (Resverlogix).
- Increases endogenous ApoA-1 production and HDL levels and thereby augment reverse cholesterol transport.
- Identified as BET inhibitor (based on GSK publication) and advertised as such on webpage (but no data have been disclosed).
- Compound structure disclosed by Resverlogix but currently not available.
- Site specific inhibitors will help to unravel function of the individual bromodomains in BETs and will further or knowledge of the design for isoform specific targeting.
RVX–208 is Selective for the Second Bromodomain of BETs

- Good selectivity for second bromodomain
- Strongest binding to BRD4(2) (130 nM)
- Selectivity in and BRD2 and BRD3 is 21 fold (ITC)
- Weaker inhibition for BRDT

<table>
<thead>
<tr>
<th>Protein</th>
<th>$K_D$ (nM)</th>
<th>$\Delta H$ (kcal/mol)</th>
<th>$\Delta S$ (kcal/mol)</th>
<th>$\Delta G$ (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRD2(1)</td>
<td>5780.3</td>
<td>± 444.17</td>
<td>-4.437</td>
<td>2.465</td>
</tr>
<tr>
<td>BRD2(2)</td>
<td>251.3</td>
<td>± 17.23</td>
<td>-4.044</td>
<td>4.423</td>
</tr>
<tr>
<td>BRD3(1)</td>
<td>4065.0</td>
<td>± 160.32</td>
<td>-4.452</td>
<td>2.655</td>
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<tr>
<td>BRD3(2)</td>
<td>194.6</td>
<td>± 12.97</td>
<td>-6.401</td>
<td>2.445</td>
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<tr>
<td>BRD4(1)</td>
<td>1141.6</td>
<td>± 46.26</td>
<td>-7.881</td>
<td>-0.042</td>
</tr>
<tr>
<td>BRD4(2)</td>
<td>134.8</td>
<td>± 8.87</td>
<td>-4.338</td>
<td>4.723</td>
</tr>
<tr>
<td>BRDT(1)</td>
<td>5405.4</td>
<td>± 236.38</td>
<td>-7.734</td>
<td>-0.789</td>
</tr>
<tr>
<td>BRDT(2)</td>
<td>636.9</td>
<td>± 38.14</td>
<td>-4.141</td>
<td>4.032</td>
</tr>
</tbody>
</table>
RVX-208 is Selective for the Second Bromodomain of BETs

- Residue differences (H433/D) provide rational for tighter binding to BRD2(2)
- Good shape complementarity with Kac site but “shelf” region not occupied by compound (DMSO binding)
- Binding mode conserved in BRD4(1) (not shown) (Reso: 1.6 Å)
Variant of RVX-208 (E25190) has different binding mode resulting in loss of selectivity for the second BRD

- E25190 is selective for BETs in ΔTm panel (46 BRDs) but selectivity for second BRDs is lost
- ITC showed that Kds are between 150 and 350 nM (BRD2 and BRD4)
- Co-Crystal Structures revealed a second binding mode that is sterically excluded for RVX-208
- In E25190 the dimethyl phenol acts as a acetyl lysine mimetic

**ITC: E25190**

<table>
<thead>
<tr>
<th>Protein</th>
<th>(K_D) (nM)</th>
<th>(\Delta H^{\text{obs}}) (kcal/mol)</th>
<th>N</th>
<th>(T\Delta S) (kcal/mol)</th>
<th>(\Delta G) (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRD2(1)</td>
<td>350 ± 16</td>
<td>-10.68 ± 0.055</td>
<td>1.03 ± 0.004</td>
<td>-2.17</td>
<td>-8.51</td>
</tr>
<tr>
<td>BRD2(2)</td>
<td>221 ± 25</td>
<td>-3.50 ± 0.044</td>
<td>1.01 ± 0.008</td>
<td>5.27</td>
<td>-8.77</td>
</tr>
<tr>
<td>BRD4(1)</td>
<td>153 ± 10</td>
<td>-11.73 ± 0.072</td>
<td>0.98 ± 0.004</td>
<td>-2.74</td>
<td>-8.99</td>
</tr>
<tr>
<td>BRD4(2)</td>
<td>218 ± 21</td>
<td>-3.463 ± 0.034</td>
<td>1.03 ± 0.007</td>
<td>5.33</td>
<td>-8.79</td>
</tr>
</tbody>
</table>
Transcription of small Subset of BET Target Genes is Affected by Inhibiting BD(2)
Clinical BET Inhibitors
(unintentional targeting)
Clinical JAK and PLK Inhibitors with Strong BET Activity
**“BioMAP” Profile**

<table>
<thead>
<tr>
<th>Human Primary Cell Type</th>
<th>Stimulation</th>
<th>Assay Readouts</th>
</tr>
</thead>
</table>
| HuVEC + PBMC co-culture | **Cocktail of two factors:**  
Superantigens  
**24-hour stimulation**  
Relevance to human disease:  
Auto-immunity (RA, IBD, COPD etc.)  
Inflammation  
Asthma/Allergy  
Oncology  
Safety | MCP-1, CD38, CD40, E-selectin, CD69, IL-8, MIG, T cell  
Proliferation, PBMC cytotoxicity, SRB |

Alison O'Mahony & Team / DiscoverX
Clinical JAK/BET and PLK/BET Inhibitors have Dual Phenotypes in Human Primary Cells
BRD inhibitors beyond BET
Targeting Bromodomain of HATs CBP/EP300

Biological Function

- General transcriptional co-activator
- Maintenance of genomic stability by affecting DNA replication and DNA repair
- Cell growth, transformation, development and neuronal plasticity/ memory formation, energy homeostasis (knockout- mouse models)
- By acetylation of non-histone proteins CBP can have a positive or negative effect on transcriptional regulation by affecting protein-protein interaction, protein-DNA interaction, nuclear retention or protein half-life

Disease relevance

- Rubinstein-Taybi syndrome (RTS): growth retardation, facial abnormalities, organ abnormalities, mental retardation, proneness to tumors
- Chromosomal translocations (MOZ, MLL) and overexpression in acute myeloid leukemia and other cancers.
- Amyotrophic lateral sclerosis, ALS, Lou Gherig’s disease, neurodegenerative disease with progressive degeneration of motor neurons in the brain and spinal cord
- Poly glutamine diseases: Spinal and Bulbar Muscular Atrophy and Huntington’s disease
iCBP122: A Chemical Probe for CBP/p300 Bromodomains
SGC-CBP30: A Chemical Probe for CBP/p300 Bromodomains

**Selectivity (ΔTₘ)**

- ΔTₘ > 9 °C
- 2 °C > ΔTₘ > 1 °C
- ΔTₘ ≤ 1 °C

**SGC-CBP30**

- CBP Kₐ = 21 nM (ITC).
- Selective over other BRD families.
- 40-fold selectivity over BRD4 (ITC).
SGC-CBP30 Binds to the Kac Site

- BDO0BO38 binds to the acetyl-lysine binding site of CBP
- Kac mimetic inhibitor (H-bond to N1168)
- Conserved water molecules present in ligand complex

- Induced fit by flipping R1173 (green carbon atoms: apo structure)
Bromodomain Probes/Leads

- Bromodomain Probes/Leads
- JQ1
- PFI-1
- (+)-JQ1
- GSK2801
- BDOBO38
- I-CBP112
- pan-BRD
- Bromosporine

**Bromodomain Probes/Leads**

- **JQ1**
  - (±)-JQ1
- **PFI-1**
- **GSK2801**
- **BDOBO38**
- **I-CBP112**
- **Bromosporine**

**nM Hits**
Open Access Probes

http://www.thesgc.org/scientists/chemical_probes

Home > Reagents & Resources > Chemical Probes

Chemical Probes

Why do we need high quality open access chemical probes?

Potent, selective and cell-permeable inhibitors of protein function ("chemical probes") are valued reagents in both fundamental and applied biological research, and they are essential for the early stages of drug discovery by allowing preclinical target validation in both academic and industrial laboratories. History shows that the pharmaceutical industry is far more likely to pursue a drug discovery programme if there are already well-characterised inhibitors with defined mechanisms of action available. However, chemical probes are not widely available because they are difficult to produce without access to skilled medicinal chemists; they are also frequently targeted to the relatively few proteins that have already been the focus of industrial drug discovery efforts and are often encumbered by intellectual property and restrictive material transfer agreements. Moreover, many of the probes currently available are inadequately characterized and nonselective, which can muddy the conclusions of experiments carried out by the research community.

One solution to this problem might be for industry to use their medicinal chemistry expertise to provide chemical probes for all potential drug targets. However, the decreasing productivity of industry requires them to apply more effort on later-stage drug development and to move away from target discovery. The situation has created a paradox: industry is increasingly dependent on academia to discover and validate new targets, yet target validation is optimally done with the use of well-characterized chemical probes, whose derivation is best done in industry.

Strategies for generating probes

Through strategic collaborations, we are applying a systematic approach to generating chemical probes for kinases and epigenetic proteins, building on the output of the SGC, which has produced most of these human proteins in purified form and has determined the three-dimensional structures of many. We have developed biophysical and biochemical assays that allow identification of hits and understanding of SAR (structure activity relationships). To generate and optimize small molecule hits, we make particular use of structure based design, including screening of fragments

Released Chemical Probes

To obtain samples of our chemical probes, please click on the appropriate link below:

- Epigenetic Probes
  - JQ1 (SGCD001) (BET Probe)
  - UNC0038 (G9a Probe)
  - PFI-1 (BET probe)
  - IXX2 (PHD2 probe)
  - GSK-J1 (KDM probe)
  - UNC1715 (L3MBT1L3 Probe)
  - SGC0846 (DOT1L Probe)
  - GSHC43 (EZH2 Probe)
  - UNC0642 (G9a/MLP Probe)
  - GSK2801 (BAZ2B/A Probe)
  - LCBP112 (CREBBP/EP300 Probe)
  - SGC-CBP30 (CREBBP/EP300 Probe)
  - UNC1999 (EZH2/1 Probe)
  - PFI-2 (SETD7 Probe)
  - A366 (G9a/GLP Probe)

Tool Compounds

To obtain samples of our tool compounds, please click on the appropriate link below:

- IXX1
  - Bromosporone
ACKNOWLEDGEMENTS

FUNDING PARTNERS
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