New Cancer Therapies on the Horizon
New Strategies for therapies of the future

Nov 20th 2020 IUPHAR Meeting
Doriano Fabbro, Cellestia Biotech
Agenda

• Immuno-oncology approaches

• Epigenetic Targets

• Drugging “undruggable targets”
  – Degraders & MS-ABPP
  – Inhibiting Ras
  – Inhibiting Transcription Factors
The cancer-immunity cycle, immune-resistant mechanisms and strategies for anti-cancer immunotherapy
Next wave IO drug development target landscape

TME immunosuppression

Discovery/Preclinical
Phase I-II development
Phase III development
Approved

“Immuno/oncometabolic” targets

Innate immune system targets

Stimulatory checkpoints
Negative checkpoints
### Next wave targets - what’s hot?

#### Commercial development heat map

<table>
<thead>
<tr>
<th>4-1BB</th>
<th>A2AR</th>
<th>Adenosine</th>
<th>Arginase</th>
<th>Arginine</th>
<th>BTLA</th>
<th>Cbl-b</th>
<th>CBP</th>
<th>CCR2</th>
<th>CCR4</th>
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<tr>
<td></td>
<td></td>
<td>CD28/80</td>
<td>CD200</td>
<td>CD206R</td>
<td>CD27</td>
<td>CD276</td>
<td>CD39</td>
<td>CD40</td>
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<td></td>
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<td>CEACAM6</td>
<td>CSF-1R</td>
<td>CXCR4</td>
<td>DPP</td>
<td>EP4</td>
<td>FoxP4</td>
<td>Galectin 3</td>
<td>Galectin 9</td>
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<td></td>
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<td>HDACs</td>
<td>HVEF</td>
<td>IAP</td>
<td>ICOS</td>
<td>IDO/ TDO</td>
<td>IDO1</td>
<td>IL15Rα</td>
<td>IL2R</td>
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<td></td>
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<td>KIR</td>
<td>KLRG1</td>
<td>LAG3</td>
<td>LIF</td>
<td>LXR</td>
<td>NKG2A</td>
<td>NLRP3</td>
<td>OX001R</td>
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<td></td>
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<td>PVRIG</td>
<td>Receptor kinases</td>
<td>RIG-I pathway</td>
<td>RORy</td>
<td>Semaphorin</td>
<td>Siglec-9</td>
<td>STAT3</td>
<td>STING</td>
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<td>TIM3</td>
<td>TLRs</td>
<td>USP7</td>
<td>VISTA</td>
<td>VSIG8</td>
<td>Wnt pathway</td>
<td></td>
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</table>

No. identified candidates per target

| 1 | 2-3 | 4-6 | 7-9 | 10-12 | >12 |

Some of the main ligands and receptors present on the surface of tumor and immune cells that are targets for approved and emerging immuno-oncology therapies.
CD47 is an immune checkpoint receptor

- CD47 is ubiquitously expressed & inhibits phagocytosis and dendritic cell maturation
- CD47 is over expressed by various cancers and poor clinical prognosis correlates with increased levels of CD47 expression
- Blocking the «dont eat me signal» could be beneficial for cancer treatment
**CD47 antagonist**

- Blocking of “Don’t Eat-me-signal” activates macrophage and DC-mediated phagocytosis as well as T-cell-mediated killing for antitumor activity
- Antibodies or SIRP1α fusion proteins have been made by Fortyseven Inc., Celgene, Surface Oncology, Trillium, ALX.
- **However, Fc-containing agents (ie Antibodies) have**
  - Significant DLTs like anemia, thrombocytopenia and leukopenia
  - Impact on normal tissues, such as liver, lung and brain – caused by high level of macrophages, expression of CD47 coupled with FcR activation
- Need for either bispecific ABs (CD47 with tumor antigen (TA) like CD19/Mesothelin etc.) or small molecules (Aurigen et al.)
## Developing Anti-CD47: mostly Abs, except Aurigen

<table>
<thead>
<tr>
<th>Name</th>
<th>Company</th>
<th>Composition</th>
<th>Target</th>
<th>Fc</th>
<th>Stage</th>
<th>Clinical Start</th>
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<tr>
<td>Hu5F9</td>
<td>Forty Seven</td>
<td>Human monoclonal antibody</td>
<td>CD47</td>
<td>IgG4</td>
<td>Phase 2</td>
<td>Aug. 2014</td>
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<td>CC90002</td>
<td>Inhibrix/Celgene</td>
<td>Human monoclonal antibody</td>
<td>CD47</td>
<td>IgG4</td>
<td>Phase 1</td>
<td>Mar. 2015</td>
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<td>SRF-231</td>
<td>Surface Oncology</td>
<td>Human monoclonal antibody</td>
<td>CD47</td>
<td>IgG4</td>
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<td>TTI-621/622</td>
<td>Trillium Therapeutics</td>
<td>Fusion protein, human SIRPa w/ active human Fc</td>
<td>CD47</td>
<td>IgG4/1</td>
<td>Phase 1</td>
<td>Jan. 2016 (May 2018)</td>
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<td>ALX-148</td>
<td>Alexo</td>
<td>High affinity IgG fusion protein w/ inactive human Fc</td>
<td>CD47</td>
<td>Inactive Fc</td>
<td>Phase 1</td>
<td>Feb 2017</td>
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<tr>
<td>OSE-172</td>
<td>OSE Therapeutics (Boehringer Ingelheim)</td>
<td>Human monoclonal antibody</td>
<td>SIRPa</td>
<td>-</td>
<td>Preclinical</td>
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<td>NI-1701 (TG-1801)</td>
<td>Novimmune</td>
<td>Human IgG bispecific</td>
<td>CD47/CD19</td>
<td>IgG1</td>
<td>Preclinical</td>
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<td>NI-1801</td>
<td>Novimmune</td>
<td>Human IgG bispecific</td>
<td>CD47/Mesothelin</td>
<td>IgG1</td>
<td>Preclinical</td>
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<td>AUR-104/105</td>
<td>Aurigene</td>
<td>Small molecule inhibitor</td>
<td>CD47</td>
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<td>Biocad</td>
<td>Human monoclonal antibody</td>
<td>CD47</td>
<td>-</td>
<td>Preclinical</td>
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<td>Synthon</td>
<td>Monoclonal antibody</td>
<td>CD47</td>
<td>-</td>
<td>Preclinical</td>
<td>-</td>
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<tr>
<td>ClinicalTrials.gov identifier</td>
<td>Phase</td>
<td>Intervention</td>
<td>Trial design</td>
<td>Estimated enrolment (n)</td>
<td>Cancer type</td>
<td>Primary end points</td>
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<tr>
<td>NCT03763149</td>
<td>I</td>
<td>Anti-CD47 antibody (IBI188)</td>
<td>Dose escalation</td>
<td>42</td>
<td>Advanced malignancies and lymphoma</td>
<td>Safety and tolerability</td>
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<tr>
<td>NCT03717103</td>
<td>I</td>
<td>Anti-CD47 antibody (IBI188) alone (Ia) or in combination with rituximab (Ib)</td>
<td>Dose escalation</td>
<td>92</td>
<td>Advanced malignancies</td>
<td>Safety and tolerability</td>
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<td>NCT02678338</td>
<td>I</td>
<td>Anti-CD47 antibody (Hu5F9-G4)</td>
<td>Dose escalation</td>
<td>20</td>
<td>Haematological malignancies</td>
<td>Tolerability</td>
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<td>NCT02216409</td>
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<td>Anti-CD47 antibody (Hu5F9-G4)</td>
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<td>Solid tumours</td>
<td>Safety and tolerability</td>
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<td>NCT03834948</td>
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<td>Anti-CD47 antibody (AO-176)</td>
<td>Dose escalation, dose expansion</td>
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<td>Advanced solid tumours</td>
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<td>NCT03013218</td>
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<td>High-affinity SIRPa fusion protein (ALX148)</td>
<td>Dose escalation</td>
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<td>Advanced solid tumours and lymphoma</td>
<td>Dose-limiting toxicity</td>
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<td>NCT03512340</td>
<td>I/Ib</td>
<td>Anti-CD47 antibody (SRF231)</td>
<td>Dose escalation, dose expansion</td>
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<td>Advanced solid and haematological cancers</td>
<td>Safety and tolerability</td>
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<td>NCT02367196</td>
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<td>Anti-CD47 antibody (CC-90002) alone or in combination with rituximab</td>
<td>Dose escalation</td>
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<td>Advanced solid and haematological malignancies</td>
<td>Tolerability and safety</td>
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<tr>
<td>NCT02663518</td>
<td>I</td>
<td>SIRPaFc (TTI-621) alone or in combination with rituximab or nivolumab</td>
<td>Dose escalation</td>
<td>260</td>
<td>Relapsed/refractory haematological and solid malignancies</td>
<td>Safety and tolerability</td>
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<tr>
<td>NCT02890368</td>
<td>I</td>
<td>SIRPaFc (TTI-621) alone or in combination with an anti-PD-1/ PD-L1 agent, pegylated IFNa2a, T-VEC or radiation</td>
<td>Non-randomized, parallel assignment</td>
<td>240</td>
<td>Solid tumours and mycosis fungoides</td>
<td>Optimal delivery regimen</td>
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<tr>
<td>NCT03248479</td>
<td>Ib</td>
<td>Anti-CD47 antibody (Hu5F9-G4) alone or in combination with azacitidine</td>
<td>Non-randomized</td>
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<td>AML and MDS</td>
<td>Safety and tolerability</td>
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<td>NCT02953509</td>
<td>Ib/Ii</td>
<td>Anti-CD47 antibody (Hu5F9-G4) in combination with rituximab</td>
<td>Single-arm, non-randomized</td>
<td>72</td>
<td>Refractory/relapsed non-Hodgkin lymphoma</td>
<td>Safety, tolerability and efficacy</td>
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<tr>
<td>NCT02953782</td>
<td>I/Ii</td>
<td>Anti-CD47 antibody (Hu5F9-G4) in combination with cetuximab</td>
<td>Single-arm, non-randomized</td>
<td>112</td>
<td>Solid tumours and advanced CRC</td>
<td>Safety, tolerability and efficacy</td>
</tr>
</tbody>
</table>
CD73 and A2AR as IO targets
Blocking Adenosine production, transport and signaling

Hypoxic condition
TGF-β1
IL-6
IL-1
Prostaglandin E1
Polyunsaturated Fatty Acid

Inflammatory cytokines
IL-4, IL-21, IL-12, IFN-α

Inosine

M2MQ
Expansion of MDSC
Treg
Tumor proliferation
Metastatic properties
Angiogenesis

NK cells
Th1 cells
Autophagy
Tumor Antigen
Cross-presentation from DC

Nucleoside Transporters

Adenosine deaminase (ADA)

Soluble CD73
Adenosine Kinase

AMP

Transcription Factors:
SP1, STAT3, Gfi1

Tumor environment

ATP
AMP

Tumor cell

Colon
Lung
Pancreas
Ovary
Breast
Melanoma
Thyroid

Wnt signaling

CD39
CD73

Transcription Factors:
SP1, STAT3, Gfi1
Immunosuppressive tumor microenvironment due to adenosine

Limitations of single agent CD73 inhibition:
- Adenosine from direct release from tumor cells
- Adenosine generation from tissue non-specific alkaline phosphatase (TNAP)

Limitations of single agent A2AR inhibition:
- Increased CD73 expression upon A2AR inhibition
- Compensatory activity of other adenosine receptors such as A2BR upon A2AR inhibition
TIM-3 and TIGIT and LAG-3

- T cells express multiple cell surface immune check points like TIM-3, TIGIT and LAG-3
- Blockage is beneficial for activating T-cells but can cause autoimmunity (or irAEs)
TIGIT/PD-L1: Rationale

• TIGIT expressed on NK and T cells inhibits the immune function by binding to PVR (CD155)
• TIGIT on tumor cells is an independent checkpoint pathway contributing to intrinsic resistance to anti-PD1.
• Co-blockade of TIGIT and PD-1/PD-L1 elicits synergistic activity in vivo
• Therefore, TIGIT combination opens all indications approved for PD-1/PD-L1
• Genentech (completed Ph-2 in NSCLC), Merck and Merck (Ph-2 in NSCLC), Arcus (completed Ph-2), BMS, Celgene, Astellas, Seattle Genetics, Iteos Therapeutics (Ph-1 studies in solid tumors ongoing)

Table 2 Clinical trials of TIM-3 inhibitors

<table>
<thead>
<tr>
<th>Year</th>
<th>Drug</th>
<th>Phase</th>
<th>Company</th>
<th>Type</th>
<th>Objective</th>
<th>ClinicalTrial.gov identifier</th>
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<tbody>
<tr>
<td>2015</td>
<td>MBG453</td>
<td>I</td>
<td>Novartis Pharmaceuticals</td>
<td>Anti-TIM-3</td>
<td>MBG453 given alone or combined with PDR001 in adult patients with advanced malignancies</td>
<td>NCT02608268</td>
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<tr>
<td>2016</td>
<td>TSR-022</td>
<td>I</td>
<td>Tesaro, Inc. (Waltham, MA, USA)</td>
<td>Anti-TIM-3</td>
<td>Dose escalation and cohort expansion study of TSR-022 in advanced solid tumors</td>
<td>NCT02817633</td>
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<td>2017</td>
<td>LY3321367</td>
<td>I</td>
<td>Eli Lilly and Company (Indianapolis, IN, USA)</td>
<td>Anti-TIM-3</td>
<td>LY3321367 alone or combined with an anti-PD-L1 antibody in advanced relapsed/refractory solid tumors</td>
<td>NCT03099109</td>
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<tr>
<td>2017</td>
<td>MBG453</td>
<td>I</td>
<td>Novartis Pharmaceuticals</td>
<td>Anti-TIM-3</td>
<td>PDR001 and/or MBG453 in combination with decitabine in AML or high-risk MDS</td>
<td>NCT03066648</td>
</tr>
</tbody>
</table>

Significant rationales for

TIGIT or TIM-3 or LAG-3 single agents and/or
TIGIT or TIM-3 or LAG-3 combo with PD-L1
## Epigenetic targets
**Writers, Erasers, Readers**

<table>
<thead>
<tr>
<th>Category</th>
<th>Epigenetic Regulators</th>
<th>Function</th>
<th>FDA-Approved Drug</th>
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</thead>
<tbody>
<tr>
<td><strong>Writers</strong></td>
<td><strong>DNMT1, 3A, and 3B</strong></td>
<td>Methylates cytosines on DNA, and mutation can lead to aberrant methylation</td>
<td>Azacitidine, decitabine</td>
</tr>
<tr>
<td></td>
<td><strong>EZH2</strong></td>
<td>Methylates histone H3K27</td>
<td>Tazemetostat</td>
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<td><strong>DOT1L</strong></td>
<td>Methylates histone H3K79</td>
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<td></td>
<td><strong>KMT2A–D, SETD2, NSD1</strong></td>
<td>Methylate histone lysines</td>
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<tr>
<td></td>
<td><strong>EP300, CREBBP</strong></td>
<td>Acetyl histone lysines</td>
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<td><strong>Erasers</strong></td>
<td><strong>TET2</strong></td>
<td>Is the first step in cytosine demethylation; is inhibited by 2-hydroxyglutarate (2-HG)</td>
<td>Azacitidine, decitabine</td>
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<tr>
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<td><strong>IDH1, IDH2</strong></td>
<td>Mutated protein produces 2-HG from isocitrate that inhibits TET2 and lysine demethylases</td>
<td>Ivosidenib, enasidenib</td>
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<tr>
<td></td>
<td><strong>HDAC1–3, 8 HDAC6</strong></td>
<td>Deacetylase removes acetyl groups from histone lysines</td>
<td>Vorinostat, belinostat, panobinostat, romidepsin</td>
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<tr>
<td></td>
<td><strong>KDM1A, KDM6A (UTX)</strong></td>
<td>Demethylates histone lysines</td>
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<tr>
<td><strong>Readers</strong></td>
<td><strong>BRD4</strong></td>
<td>Bromodomain proteins read acetyl groups on histone lysines</td>
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</tr>
<tr>
<td></td>
<td><strong>CBX family, CHD1</strong></td>
<td>Chromodomain proteins read methyl groups</td>
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</tbody>
</table>
### Epigenetic targets
**Movers, Shapers, Insulators**

<table>
<thead>
<tr>
<th>Category</th>
<th>Epigenetic Regulators</th>
<th>Function</th>
<th>FDA-Approved Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Movers</strong></td>
<td>ARID1A, ARID1B, ARID2 SMARCA2, SMARCA4, SMARCB1, CHD1</td>
<td>Proteins in the chromatin remodeling complex use ATP to move nucleosomes away from DNA; loss-of-function mutations common in cancer</td>
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<tr>
<td><strong>Shapers</strong></td>
<td>HIST1H1B, HIST1H1C, HIST1H3B, H3F3A, H3F3B</td>
<td>Structural histone proteins acquire mutations that can be oncogenic</td>
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<tr>
<td><strong>Insulators</strong></td>
<td>CTCF, STAG2, RAD21, CHD8</td>
<td>Normal binding to CTCF sites on DNA defines and protects gene neighborhoods from inappropriate expression</td>
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</table>
PRMT5 catalyzes the formation of symmetric dimethyl arginine (SDMA) in many cellular proteins.

SDMA regulates cancer relevant proteins and pathways.

PRMT5 is key regulator of cellular splicing and its overexpression signifies poor prognosis.
Potential Therapeutic Application of PRMT5i Synthetic Lethality in Spliceosome Mutant Cancers

- Splicing of mRNA precursors is a key step in regulating expression of many genes.
- Spliceosome proteins are rendered functional by post-translational methylation by PRMT5.
- High frequency of hotspot mutations in the spliceosome proteins SF3B1, SRSF2, and/or U2AF1 have been reported in several cancers (35-40% of MDS, 5-18% in CLL, 5-25% in AML, 14-29% in uveal melanoma).
- Functional redundancy exists among members of the spliceosome complex.
- PRMT5 inhibition potentiates synthetic lethality in spliceosome mutant cancers.
- Approach is currently being evaluated in clinical trials.

Nat Rev Cancer 2016 17; 413-430; Nat Struct Mol Biol 2019; 26, 999-1012
Potential Therapeutic Application of PRMT5i
Turning cold tumors to hot

- Epigenetic changes are required for the expression of ISG genes in tumor cells leading to tumor IFN-driven resistance.
- PRMT5i is reported to inhibit expression of resistant genes in tumor cells.
- Conversely, PRMT5i allows uninterrupted IFNG signaling in immune cells.
- PRMT5i would phenocopy tumor IFNGR ablation while allowing IFNG-driven favorable immune response.
- Expected to enhance anti-tumor efficacy of anti-PD1 antibody. Combination is currently being evaluated in clinical trials.

## PRMT5i: Competitive Landscape

<table>
<thead>
<tr>
<th>Drug/Company name</th>
<th>Type</th>
<th>Highest Dev. Status</th>
</tr>
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<tbody>
<tr>
<td>GSK-3326595/ GSK and Epizyme Inc</td>
<td>Substrate competitive</td>
<td>Phase 1/2 Clinical</td>
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<tr>
<td>JNJ-64619178/Janssen Research &amp; Development LLC</td>
<td>SAM competitive</td>
<td>Phase 1 Clinical</td>
</tr>
<tr>
<td>PF-06939999/Pfizer Inc.</td>
<td>SAM competitive</td>
<td>Phase 1 Clinical</td>
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<tr>
<td>PRT-811/Prelude Therapeutics Inc.</td>
<td>Unknown</td>
<td>Phase 1 Clinical</td>
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<tr>
<td>PRT-543/Prelude Therapeutics Inc.</td>
<td>Unknown</td>
<td>Phase 1 Clinical</td>
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<tr>
<td>Cancer Therapeutics CRC/Merck MSD</td>
<td>-</td>
<td>Discovery</td>
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<tr>
<td>CT-300/Celleron Therapeutics Ltd</td>
<td>-</td>
<td>Discovery</td>
</tr>
<tr>
<td>Bayer Pharma</td>
<td>-</td>
<td>Discovery</td>
</tr>
<tr>
<td>Argonaut Therapeutics Ltd</td>
<td>-</td>
<td>Discovery</td>
</tr>
</tbody>
</table>

- GSK has initiated Phase 2 trials in MDS and AML; Combination with 5-azacitidine and Pembrolizumab
Novel paradigm in drug development using the UPS

### Molecular Glues
- Modulate E3 surface to recognize neo-substrate

### PROTACs
- Tethering to enforce proximity to E3

### Destabilizer
- Destabilize target to make it E3 degradable

<table>
<thead>
<tr>
<th>Type of molecule</th>
<th>monovalent</th>
<th>heterobifunctional</th>
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<tbody>
<tr>
<td>Design principles</td>
<td>Non-rational</td>
<td>Modular: ligand$^{E3}$-linker-ligand$^{POI}$</td>
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<tr>
<td>Examples</td>
<td>IMiDs (clinical reality)</td>
<td>ARV-110, ARV-471 (in clinical trials)</td>
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## Pros and Cons

<table>
<thead>
<tr>
<th>PROTAC</th>
<th>Molecular Glue</th>
<th>Destabilizers</th>
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</thead>
<tbody>
<tr>
<td><strong>Bi-functional molecules bind to E3 ligase and a single protein simultaneously, causing proximity induced degradation</strong>&lt;br&gt;Amenable to any protein with a known binder. Optimization of linkers, ligands and couplings very well suited for combinatorial probing. <strong>Path to ID’ing degraders &amp; optimizing efficacy, differentiation and validation is well defined</strong>&lt;br&gt;Wide variety of targets already demonstrated to be amenable for PROTAC degradation</td>
<td><strong>Bind to E3 ligases and degrade recruited protein(s) via a ternary complex</strong>&lt;br&gt;Smaller and more drug-like molecules e.g. IMIDs and indusulam</td>
<td><strong>Monovalent molecules that bind to a protein and cause destabilization and degradation</strong>&lt;br&gt;Small and drug like – derived from recognizable inhibitor templates, often with very small (single atom) changes&lt;br&gt;Target biased: bromodomains, nuclear receptors and kinases all shown to be capable of being degraded&lt;br&gt;No rational way – yet – to design destabilizers. Examples so far found serendipitously during SAR campaigns for inhibitors</td>
</tr>
<tr>
<td>Molecules tend to be larger and less drug-like; Beyond Ro5 with implications for achieving good PK/ADME/tox</td>
<td>No rational way to design glues, and no way to predict which protein(s) they will degrade Pure screening and empiricism; difficult to predict pleotropy</td>
<td>Validation of therapeutic potential &amp; safety of novel degraded proteins can be a massive effort</td>
</tr>
</tbody>
</table>
Glue-degrader
IMiD-Induced CRBN-Dep. Degradation of Neosubstrates

DOI: 10.1021/acs.biochem.8b01307
Biochemistry 2019, 58, 861–864
Glue degrader
Structures and clinic for FDA-approved Imids

- Multiple myeloma (+ dexamethasone)
- Erythema nodosum leprosum

- Multiple myeloma (+ dexamethasone)
- del(5q) myelodysplastic syndrome
- Relapsed mantle cell lymphoma

IMiDs

- Broad anti-tumor effect in cancer cell lines and primary AML cells (sub-nanomolar potency)
- Induces degradation of GSPT1

- (Phase 1/2) Unresectable hepatocellular carcinoma (+ nivolumab)
- (Phase 1/2) Chronic lymphocytic leukemia (+ ibrutinib & obinutuzumab)
- (Phase 1) Relapsed/refractory diffuse large B-cell lymphoma (+ obinutuzumab)

THALOMID®
(Thalidomide)
POMALYST®
(Pomalidomide)
REVLIMID®
(Lenalidomide)
CC-885
CC-220
CC-122

DOI: 10.1021/acs.biochem.8b01307
Biochemistry 2019, 58, 861-864
Glue degrader
Expanding on Glue degrader

Auxin
TIR1

Lenalidomide
CRBN

NRX-1933
B-TrCP

(R)-CR-8
DDB1

Tan et al., 2007, Nature
Sievers et al., 2018, Science
Simonetta et al., 2019, Nat. Comm.
Słabicki et al., 2019, Nature.
Bifunctional degrader approach for protein kinases or other POIs

Destroy the kinase target rather than inhibiting it
<table>
<thead>
<tr>
<th>Target(s)</th>
<th>Ligase(s) (References)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABC50</td>
<td>CRBN (Cie slak et al., 2019)</td>
</tr>
<tr>
<td>ALK</td>
<td>CRBN (Powell et al., 2018; Zhang et al., 2018)</td>
</tr>
<tr>
<td>AR</td>
<td>MDM2 (Schneekloth et al., 2008)</td>
</tr>
<tr>
<td>AURKA/B</td>
<td>CRBN (Huang et al., 2018)</td>
</tr>
<tr>
<td>BCL2</td>
<td>CRBN (Wang et al., 2019)</td>
</tr>
<tr>
<td>BCL6</td>
<td>CRBN (McCoul et al., 2018)</td>
</tr>
<tr>
<td>BCR-ABL</td>
<td>IAP; CRBN; VHL (Demizu et al., 2016; Lai et al., 2016)</td>
</tr>
<tr>
<td>BRAF</td>
<td>CRBN (Chen et al., 2019a)</td>
</tr>
<tr>
<td>BRD2, BRD3, BRD4</td>
<td>CRBN; VHL (Lu et al., 2015; Winter et al., 2015; Zengerle et al., 2015)</td>
</tr>
<tr>
<td>BRD7, BRD9</td>
<td>VHL (Zoppi et al., 2019)</td>
</tr>
<tr>
<td>BTK</td>
<td>CRBN (Buhimschi et al., 2018; Sun et al., 2018; Zorba et al., 2018)</td>
</tr>
<tr>
<td>c-ABL</td>
<td>CRBN; VHL (Lai et al., 2016)</td>
</tr>
<tr>
<td>CDK4/6</td>
<td>CRBN (Jiang et al., 2019; Winter et al., 2015; Zengerle et al., 2015)</td>
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<tr>
<td>CDK9</td>
<td>CRBN (Olson et al., 2018; Robb et al., 2017)</td>
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<tr>
<td>cIAP1</td>
<td>IAP (Itoh et al., 2012)</td>
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<td>CRABP I/II</td>
<td>IAP (Itoh et al., 2012; Itoh et al., 2010)</td>
</tr>
<tr>
<td>CRBN</td>
<td>VHL; CRBN (Steinebach et al., 2019; Steinebach et al., 2018)</td>
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<tr>
<td>EGFR</td>
<td>VHL (Burslem et al., 2018a)</td>
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<td>ER</td>
<td>IAP (Demizu et al., 2012; Itoh et al., 2011)</td>
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<tr>
<td>ERK1/2</td>
<td>CRBN (Lebraud et al., 2016)</td>
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<tr>
<td>ERRa</td>
<td>VHL (Bondeson et al., 2015)</td>
</tr>
<tr>
<td>FAK</td>
<td>VHL (Cromm et al., 2018)</td>
</tr>
<tr>
<td>FKBP12</td>
<td>CRBN (Winter et al., 2015)</td>
</tr>
<tr>
<td>FLT3</td>
<td>VHL; CRBN (Burslem et al., 2018b; Huang et al., 2018)</td>
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<tr>
<td>GSPT1</td>
<td>CRBN (Matyskiela et al., 2016)</td>
</tr>
<tr>
<td>HCV</td>
<td>NS3/4A CRBN (de Wispelaere et al., 2019)</td>
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<tr>
<td>HDAC6</td>
<td>CRBN (Yang et al., 2018)</td>
</tr>
<tr>
<td>HER2</td>
<td>VHL (Burslem et al., 2018a)</td>
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<tr>
<td>IRAK4</td>
<td>VHL (Nunes et al., 2019)</td>
</tr>
<tr>
<td>ITK</td>
<td>CRBN (Huang et al., 2018)</td>
</tr>
<tr>
<td>Mcl1</td>
<td>CRBN (Wang et al., 2019)</td>
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<tr>
<td>MDM2</td>
<td>CRBN (Li et al., 2019b)</td>
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<tr>
<td>p38a/delta</td>
<td>VHL; CRBN (Bondeson et al., 2018; Smith et al., 2019)</td>
</tr>
<tr>
<td>PARP1</td>
<td>MDM2 (Zhao et al., 2019)</td>
</tr>
<tr>
<td>PCAF/GCN5</td>
<td>CRBN (Bassi et al., 2018)</td>
</tr>
<tr>
<td>PIRIN</td>
<td>CRBN (Chessum et al., 2018)</td>
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<tr>
<td>PTK2/B</td>
<td>VHL; CRBN (Popow et al., 2019)</td>
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<tr>
<td>RAR</td>
<td>IAP (Itoh et al., 2011)</td>
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<tr>
<td>RIPK2</td>
<td>VHL (Bondeson et al., 2015)</td>
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<tr>
<td>Rpn13</td>
<td>CRBN (Song et al., 2019)</td>
</tr>
<tr>
<td>SGK3</td>
<td>VHL (Tovell et al., 2019b)</td>
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<td>sirtuin-2</td>
<td>CRBN (Schiedel et al., 2018)</td>
</tr>
<tr>
<td>SMAD3</td>
<td>VHL (Wang et al., 2016)</td>
</tr>
<tr>
<td>SMARCA2/4</td>
<td>VHL (Farnaby et al., 2019)</td>
</tr>
<tr>
<td>TACC3</td>
<td>IAP (Ohoka et al., 2014)</td>
</tr>
<tr>
<td>tau</td>
<td>CRBN (Silva et al., 2019)</td>
</tr>
<tr>
<td>TBK1</td>
<td>VHL (Creel et al., 2018)</td>
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<tr>
<td>TEC</td>
<td>CRBN; VHL; IAP (Zorba et al., 2018; Huang et al., 2018)</td>
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<tr>
<td>TRIM24</td>
<td>VHL (Gechijian et al., 2018)</td>
</tr>
<tr>
<td>TrkC</td>
<td>CRBN (Zhao and Burgess, 2019b)</td>
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<tr>
<td>ULK1</td>
<td>CRBN (Huang et al., 2018)</td>
</tr>
<tr>
<td>VHL</td>
<td>VHL (Maniacci et al., 2017)</td>
</tr>
</tbody>
</table>

List of present targets for non-peptidic PROTACs

Two thus far in clinical trials

https://doi.org/10.1016/j.molcel.2020.01.010
## ARVINAS

<table>
<thead>
<tr>
<th>Program/Target</th>
<th>Discovery</th>
<th>Lead Optimization</th>
<th>IND Enabling</th>
<th>Phase 1</th>
<th>Arvinas Owned</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locally Advanced or Metastatic ER+/HER2- Breast Cancer</td>
<td>ARV-471 [Estrogen Receptor]</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Additional Oncology Indications</td>
<td>e.g., CRC, NSCLC [Various Undisclosed]</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>Tauopathies</td>
<td>e.g., PSP(^2) [Tau]</td>
<td>✓</td>
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<td>✓</td>
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<tr>
<td>Synucleinopathies</td>
<td>e.g., MSA(^3), Parkinson’s [α-synuclein]</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Additional Neurology Indications</td>
<td>Various [Undisclosed]</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>
ARV-110 degraded 95% to 98% of AR in multiple cell lines typically used in prostate cancer research, including VCaP cells

- **DC$_{50}$ in VCaP = 1 nM**, Dmax@4 hrs
- **60x** more potent than enzalutamide

**ARV-110**

### Androgen Receptor (AR) Activity Drives Prostate Cancer

- Current agents work by decreasing androgen levels (abiraterone) or blocking androgen binding to AR (enzalutamide)
- **15-25%** of patients never respond to abiraterone or enzalutamide (**intrinsic resistance**)
- **Resistance mechanisms** to abiraterone and enzalutamide include:
  - AR gene amplification (**40-60%** of patients)
  - AR gene enhancer amplification (**>70%** of patients)
  - AR point mutations (**~15%** of patients)
  - Intra-tumoral androgen production

### PROTAC® Degrader ARV-110

- Highly selective degrader of AR; DC$_{50}$ = 1 nM
- In preclinical models, overcomes resistance mechanisms to enzalutamide and abiraterone
- Not brain penetrant
- First-in-class AR degrader being tested in men with metastatic castration-resistant prostate cancer who have progressed on standards of care (enzalutamide, abiraterone)
- **Phase 1 clinical trial initiated 1Q19**
- Received FDA “Fast Track” designation in May 2019

---

![Graph showing proliferation cell/titerGlo (RLU) vs. compound concentration (nM)](image-url)
ARV-110 Inhibits AR-Dependent Tumor Growth in Xenograft Models with Oral, Daily Dosing

- 10 patients with mCRPC treated across three dose levels
- At doses up to 280 mg with an acceptable safety profile
- PK dose-proportional increase in exposure
- PSA and RECIST responses and PD/molecular marker planned in 1st half 2020 at major medical conference
ARV-471: ER Degrader for Patients with Locally Advanced or Metastatic Breast Cancer

Breast cancer is the second most common cancer in women\(^1\)

- ~268,000 women are expected to be diagnosed with invasive breast cancer in the US in 2019\(^1\)
- Metastatic breast cancer accounts for ~6% of newly diagnosed cases\(^2\)
- 80% of newly diagnosed breast cancers are estrogen receptor (ER) positive\(^3\)
- Fulvestrant has validated the relevance of ER degradation in breast cancer
- After 6 months of fulvestrant treatment, up to 50% of ER baseline levels remain\(^4\)

PROTAC\(^\circledast\) Degrader ARV-471

- ARV-471 is a potent degrader (DC\(_{50} = 1.8\) nM) of the estrogen receptor, which is in development for the treatment of patients with ER+ locally advanced or metastatic breast cancer
- **Phase 1 clinical trial initiated 3Q2019**
- After Phase 1 dose escalation, a Phase 1b trial in combination with CDK4/6 inhibitor is planned

\(^1\) American Cancer Society; \(^2\) American Cancer Society; \(^3\) National Cancer Institute, Hormone Therapy for Breast Cancer; \(^4\) National Cancer Institute, Hormone Therapy for Breast Cancer
Orally Dosed ARV-471 Shrinks Tumors and Robustly Degrades ER in MCF7 Xenografts

---

**Graph**

- **X-axis**: Days of dosing
- **Y-axis**: Mean Tumor volume (mm³)
- **Legend**:
  - Vehicle
  - ARV-471, 3 mpk
  - ARV-471, 10 mpk
  - ARV-471, 30 mpk
- **Data Points**:
  - TG (18 hours post last dose): 85% (Vehicle), 98% (3 mpk), 124% (10 mpk), 124% (30 mpk)

**Table**

<table>
<thead>
<tr>
<th>Dose (po, qd)</th>
<th>Mean AUC_{0-24} (ng*hr/ml)</th>
<th>Mean C_{max} (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 mpk</td>
<td>658</td>
<td>84</td>
</tr>
<tr>
<td>10 mpk</td>
<td>2538</td>
<td>312</td>
</tr>
<tr>
<td>30 mpk</td>
<td>5717</td>
<td>962</td>
</tr>
</tbody>
</table>

**Western Blot**

- **Vehicle**
- **ARV-471 3 mpk**
- **ARV-471 10 mpk**
- **ARV-471 30 mpk**

**ER Reduction**

- 3 mpk: 95%
- 10 mpk: 97%
- 30 mpk: 94%
ARV-471: Phase 1 Study: FIH 08-2019

Design:
- “3 + 3” dose escalation; starting dose = 30 mg orally, once daily (po, qd) with food
- Dose increases dependent on toxicities: range 25% (if 1 DLT in 6 pts) to 100% (≤Grade 1 Adverse Events)

Key Entry Criteria:
- ER+/HER2- advanced breast cancer
- At least two prior endocrine therapies in any setting, and a CDK4/6 inhibitor
- Up to three prior cytotoxic chemotherapy regimens

Key Objectives:
- Maximum Tolerated Dose/ Recommended Phase 2 Dose/Safety
- Pharmacokinetics
- Anti-tumor activity (RECIST, CBR)
- Biomarkers

Biomarkers:
- ER gene (ESR1) mutational status in ctDNA and/or tumor tissue
- ER, Progesterone Receptor and Ki-67 levels in pre- and post-treatment tumor biopsies in patients with accessible tumor tissue

Accumulation occurs between Day 1 and Day 15 (30 mg)
The undruggable proteome

Over 90% of proteins do not have known pocket that small molecule can bind

Identify target binding pockets «druggable hotspots» across the entire proteome
MS-Activity Based Protein Profiling

Reactivity based probes for mapping ligandable «druggable hotspots» across the entire proteome

1. Avidin enrichment
2. Tryptic digest
3. TEV digest
TPD
Inventing induced proximity druggable mechanisms

- Dub targeting Chimeras (DubTACs)
- Phosphatase targeting Chimeras (PhosphaTACs)
- Acetyltransferase targeting Chimeras (AATACs)
- Deacetylase targeting Chimeras (DATACs)
- Conformation targeting Chimeras (DubTACs)
- Etc…

| Table 2. Summary of the Advantages and Disadvantages of Described Methods of Targeted Protein Degradation |
|---------------------------------------------------------------|---------------------------------------------------------------|
| Method            | Advantages                                                                                                                                                                                                 | Disadvantages                                                                                                                                               |
| PROTACs           | Catalytic; reversible; endogenous wild-type and mutant targets; paralog-selective; in vivo applications.                                                                                                   | Lengthy SAR analyses of linker and target ligands; high MW and not "rule-of-5" compliant.                                                                   |
| Molecular glue    | Catalytic; reversible; endogenous wild-type and mutant targets; paralog-selective; in vivo efficacy in humans with some BBB-permeable.                                                                 | Challenging to identify/synthesize prospectively; specificity issues.                                                                                      |
| Trim-Away         | Endogenous targets.                                                                                                                                                                                       | Limited by endogenous levels of Trim21, Ab access to cytosolic but not nuclear targets; Ab specificity to target.                                         |
| CMA                | No dependency on ubiquitination machinery for targeting to lysosome.                                                                                                                                       | Ectopic expression of engineered constructs; non-catalytic; only cytosolic targets.                                                                      |
| AID                | Reversible; versatile.                                                                                                                                                                                    | Ectopic expression of F-box receptor; 7 kDa target modification; no in vivo application; leaky degradation in absence of auxin.                      |
| dTAG               | Catalytic; reversible; in vivo applications.                                                                                                                                                            | 12 kDa target modification.                                                                                                                                 |
| IKZF3 3i dissociant | Catalytic; reversible; in vivo applications including CNS; rodent IKZF3 not targeted.                                                                                                                   | 3 kDa target modification best at C terminus; not all targets degraded.                                                                                     |
| SMAS               | Reversible.                                                                                                                                                                                              | Only for newly synthesized protein.                                                                                                                       |
| HaloTag HyT       | Tagged ORFs and PROTAC commercially available; in vivo applications.                                                                                                                                   | Covalent 33 kDa target modification; non-catalytic.                                                                                                         |
| Nanobodies: deGradFP and AID nanobody | Suitable for GFP-tagged ORFs available commercially.                                                                                                                                                    | Target modification; substrate receptor engineering.                                                                                                      |

The broad applicability of emerging modalities such as AUTACs, ENDTACs/LYTACs, and RIBOTACs briefly mentioned in this Review remains to be determined and hence is not summarized here. SAR, structure-activity relationship; SMs, small molecules; BBB, blood-brain barrier; CNS, central nervous system; ORFs, open reading frames.
Ras inhibitors

Effectors

RAS-GDP (off)

GAP complex

RAS-GTP (on)

GEF complex

GTP

GEF bound
Switch 1
High (µM) Affinity for GNP

Switch II
GDP bound
Low (µM) Affinity for GNP

SOS1 (927-946)

PI3K complex

GDP

P_i

RAF complex

Effectors
Ras vulnerabilities for anti-Ras strategies

Clint A. Stalnecker, and Channing J. Der


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K-Ras inhibitors targeting the Cys12

Table 1 | Companies with RAS inhibitor in clinical development

<table>
<thead>
<tr>
<th>Developer</th>
<th>Molecule</th>
<th>Description</th>
<th>Clinical status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amgen, Carmot Therapeutics</td>
<td>AMG-510</td>
<td>KRAS&lt;sup&gt;G12C&lt;/sup&gt; inhibitor</td>
<td>Phase 1/2</td>
</tr>
<tr>
<td>Mirati Therapeutics</td>
<td>MRTX849</td>
<td>KRAS&lt;sup&gt;G12C&lt;/sup&gt; inhibitor</td>
<td>Phase 1/2</td>
</tr>
<tr>
<td>Johnson &amp; Johnson, Wellspring</td>
<td>JNJ-74699157 (formerly</td>
<td>KRAS&lt;sup&gt;G12C&lt;/sup&gt; inhibitor</td>
<td>Phase 1</td>
</tr>
<tr>
<td>Biosciences</td>
<td>ARS-3248</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eli Lilly</td>
<td>LY3499446</td>
<td>KRAS&lt;sup&gt;G12C&lt;/sup&gt; inhibitor</td>
<td>Phase 1/2</td>
</tr>
<tr>
<td>Moderna, Merck</td>
<td>V941 (mRNA-5671)</td>
<td>Lipid-nanoparticle-formulated mRNA-based vaccine targeting</td>
<td>Phase 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KRAS&lt;sup&gt;G12D&lt;/sup&gt;, KRAS&lt;sup&gt;G12V&lt;/sup&gt;, KRAS&lt;sup&gt;G13D&lt;/sup&gt; and KRAS&lt;sup&gt;G12C&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Revolution Medicines</td>
<td>NA</td>
<td>Inhibitors of KRAS&lt;sup&gt;G12C&lt;/sup&gt;, KRAS&lt;sup&gt;G13C&lt;/sup&gt;, KRAS&lt;sup&gt;G12D&lt;/sup&gt; and NRAS&lt;sup&gt;G12C&lt;/sup&gt;</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Mirati Therapeutics</td>
<td>NA</td>
<td>KRAS&lt;sup&gt;G12D&lt;/sup&gt; inhibitor</td>
<td>Preclinical</td>
</tr>
</tbody>
</table>

NA, not available. Sources: ClinicalTrials.gov, company websites, Cancer.gov.
How Ras talks to Raf

- ATP-bound BRAF and ADP-bound MEK
- Ser365 and Ser729 phosphorylation
- RAS binding
- 14-3-3 release from BRAF and p5365 segment
- Plasma membrane anchoring
- 14-3-3 rearrange to bind p5729 sites of BRAF
- Dimerization
Targeting Myc in Cancer

Targeting Myc transcription: BRD4, CDK7, CDK9, p300/CBP-BRD
Targeting Myc mRNA translation: mTOR, CPEBP
Targeting Myc stability: USP28, USP36, AURKA, PLK1, Omomyc, GTI19077
Design of OMOMYC

Cancer cell
- Myc → Proliferation
- Max → Transformation
- DNA target gene → Apoptosis
- Max → Cell cycle arrest
- DNA target gene → Differentiation

Cancer cell treated with Omomye
- OMO Myc
- OMO Max
- OMO OMO
- DNA target gene
- DNA target gene
- Cell cycle arrest
- Death of tumor cells

Dox treatment
- Omo/Ras multiple cycles
- Omo/Ras 1 cycle

% survival

weeks post infection
Discovery of Hit compound (A0) that directly targets the intrinsically disordered protein c-Myc

A

- 2 Conformations
- 3 Binding Sites

Direct Binding Assays
- CD
- SPR
- NMR
- MD simulations

PP Interference Assays
- SPR Competitive Experiments
- Cross-lignding Experiments

Cell Based Assays
- Cytotoxicity Tests
- Cell Cycle Analysis
- c-Myc Dependent Transcription

Conformations Generation and Binding Sites Prediction
Virtual Screening
Experimental Validation

PKUMDML - A0

- A0 Hit compound identification (A)
- Predicted A0 binding sites in c-Myc (B)
GT19077 selectively degrades c-Myc protein and inhibits cell proliferation in HL-60 cells

<table>
<thead>
<tr>
<th>Compounds</th>
<th>C-Myc degradation (ELISA)</th>
<th>Cell proliferation (CTG)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>HL-60</td>
<td>TF-1 (GM-CSF)</td>
</tr>
<tr>
<td>GT19077</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IC50(µM)</td>
<td>0.39</td>
<td>2.30</td>
</tr>
</tbody>
</table>

### B

- **HL60**
  - DMSO 0.1 0.2 0.5 1.0 2.0 5.0
  - GT19077 (µM)
  - c-Myc
  - GAPDH

- **TF-1 (GM-CSF)**
  - DMSO 0.1 0.2 0.5 1.0 2.0 5.0
  - GT19077 (µM)
  - c-Myc
  - GAPDH

- **NK-92 (IL-2)**
  - Ctrl
  - GT19077
  - tofacitinib
  - 500nM 1uM 2uM 1uM
  - c-Myc
  - GAPDH

GT19077 selectively degraded c-Myc in HL-60 cells with much less activity in GM-CSF stimulated TF-1 or IL-2 stimulated NK-92 cells (erythroblast and natural killer cells, respectively.)

GT19077 also selectively inhibited proliferation of HL-60 cells with less potency in GM-CSF stimulated TF-1 cells
In vivo target engagement of GT19077 with c-Myc in HL-60 and Ramos xenograft tumors (SC)

**GT19077 in vivo target inhibition**

<table>
<thead>
<tr>
<th>GT19077 Doses</th>
<th>Plasma concentration (ng/ml) 0.5 hr post last Rx (3 day Rx)</th>
</tr>
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<tbody>
<tr>
<td>10mpk</td>
<td>1012±333.75</td>
</tr>
<tr>
<td>20mpk</td>
<td>1996±501.48</td>
</tr>
<tr>
<td>40mpk</td>
<td>3182±474.10</td>
</tr>
</tbody>
</table>

**c-Myc degradation in HL60 tumors**

- **GT19077**
  - Vehicle
  - 20mpk
  - 40mpk

**c-Myc degradation Ramos tumors**

- **GT19077**
  - Vehicle
  - 40mpk

- **GAPDH**

- **GT19077 demonstrates PK-dependent c-Myc degradation in HL60 and Ramos xenograft tumors**
Targeting Myc Expression Through G-Quadruplexes

**MYC Promoter:**

- Not on coding strand, not highly conserved
- Many quadruplex ligands are known
- It is difficult to identify molecules that *selectively* bind to quadruplexes of interest

Balasubramanian, Burrows, Hurley, Neidle, Yang, and others
Preparation of an Isotopically labeled DC34 Facilitates an NMR Structure

\[
\begin{align*}
\text{DMAP} & \rightarrow \text{R} \rightarrow \text{In(DTT)}_2 \rightarrow \text{Tolue} \rightarrow \text{HCl} \rightarrow \text{HO\_formaldehyde} \rightarrow \text{HO\_formaldehyde} \\
70-85\% & \rightarrow 35-45\% \rightarrow 75-85\%
\end{align*}
\]

\[\text{C-enriched starting materials}\]

Isotopically labeled probe enables straightforward residue assignment (Haf Filtered NOESY) \(\rightarrow\) additional restraints

\[\text{Detail of 3' binding site}\]

- Tails move to accommodate binding (2 sites)
- Ligand: 3-dimensional conformation
- Affinity: hydrogen bonding, cation-\(\pi\), F-bonding

Evalutation of DC-34 in a Mouse Tumor Xenograft Model

Day 0 Day 5

<table>
<thead>
<tr>
<th>Vehicle (1X PBS)</th>
<th>DC34-Free Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYC (57kD)</td>
<td></td>
</tr>
<tr>
<td>Vinculin (124kD)</td>
<td></td>
</tr>
</tbody>
</table>

Plasma:
- \(C_{max} = 270\, \text{nM}, 30\, \text{min}\)
- \(T_{1/2} = 24\, \text{hours}\)

Tumor:
- 100-225 pmol/L
**Rapalogs vs TORKi vs Rapalink**

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**mTORC1**
- Rapalogs
- Rapalinks
- S6Ks
- 4E-BPs
- Others (ULK1, etc.)
- PRAS40
- Deptor

**mTORC2**
- TORKi
- mTOR
- Rictor
- Protor1/2
- Sin1
- Deptor
- AKT
- SGK
- PKC

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MW = 1784

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Yang et al. 2013, Nature 47: 217

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MW = 1784
RapaLink-1 is a non-traditional drug-like molecule with exceptional in vivo efficacy.

MW = 1784

mTOR (A2034V) mTOR (M2327I)