The BET Bromodomain inhibitor OTX015 affects pathogenetic pathways in preclinical B-cell Tumor models and synergizes with targeted drugs

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Overview on OTX015 in lymphoid tumors

• Introduction

• BET Bromodomain inhibitors as single agent in mature B-cell lymphomas: activity and mechanisms of action of OTX015

• Combination studies

• Possible biomarkers

• Conclusions
Haematological cancers are among the commonest cancers in adults.

### Estimated New Cases

<table>
<thead>
<tr>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate</td>
<td>220,803</td>
</tr>
<tr>
<td>Lung &amp; bronchus</td>
<td>115,619</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>68,093</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>56,323</td>
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<tr>
<td>Melanoma of the skin</td>
<td>42,673</td>
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<tr>
<td>Non-Hodgkin lymphoma</td>
<td>39,853</td>
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<tr>
<td>Kidney &amp; renal pelvis</td>
<td>38,273</td>
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<tr>
<td>Oral cavity &amp; pharynx</td>
<td>32,670</td>
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<tr>
<td>Leukemia</td>
<td>30,903</td>
</tr>
<tr>
<td>Liver &amp; intrahepatic bile duct</td>
<td>25,510</td>
</tr>
<tr>
<td>All Sites</td>
<td>946,200</td>
</tr>
</tbody>
</table>

### Estimated Deaths

<table>
<thead>
<tr>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung &amp; bronchus</td>
<td>86,383</td>
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<tr>
<td>Prostate</td>
<td>27,543</td>
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<tr>
<td>Colon &amp; rectum</td>
<td>26,100</td>
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<tr>
<td>Pancreas</td>
<td>20,713</td>
</tr>
<tr>
<td>Liver &amp; intrahepatic bile duct</td>
<td>17,030</td>
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<tr>
<td>Leukaemia</td>
<td>14,213</td>
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<tr>
<td>Esophagus</td>
<td>12,603</td>
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<tr>
<td>Urinary bladder</td>
<td>11,513</td>
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<tr>
<td>Non-Hodgkin lymphoma</td>
<td>11,680</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>9,073</td>
</tr>
</tbody>
</table>

< 20 years of age

Siegel et al, CA: Cancer 2015

Stewart & Wild eds. World Cancer Report 2014
Still too many patients die due to lymphoma

Need of novel therapies
Very complex networks
Multiple interconnections
High redundancy
Flexibility/Reprogramming

Many altered pathways = Many therapeutic targets

modified from Testoni et al, Ann Oncology 2015
Many altered pathways = Many therapeutic targets

Multiple components are deregulated in cancers
Multiple targets
Many drugs
Need of combinations

modified from Testoni et al, Ann Oncology 2015
Epigenome as a therapeutic target

Epigenome alterations, such as abnormal DNA methylation and chromatin structure, are common in cancers.

Chromatin modifications are fundamental for the regulation of:
- transcription
- DNA repair
- DNA replication

Importantly, chromatin modifications can be reversible.
Different classes of proteins are involved in transcription regulation

Dawson et al, NEJM 2012
Different classes of proteins are involved in transcription regulation

Writers

EZH2

Erasers

HDAC

Dawson et al, NEJM 2012
Different classes of proteins are involved in transcription regulation

- **Writers**: EZH2
- **Erasers**: HDAC
- **Readers**: BET Bromodomain proteins

Dawson et al, NEJM 2012
Bromodomain and extra-terminal (BET) protein family

- Nuclear proteins
- Widely expressed
- Recruit transcription factors (P-TEFb) to acetylated chromatin

Mod. From Chiang, Biol Rep 2009
OTX015 Inhibits Binding of BET Bromodomain (BRD) Proteins to Acetylated Histones
Wide anti-proliferative activity in lymphomas

G1 arrest and decreased S-phase
Induction of a senescence-like phenotype
In vivo anti-tumor activity in lymphoma

![Graph showing tumor volume over time for CTRL and OTX015 (50mg/Kg) treatments. The graph indicates a significant decrease in tumor volume for OTX015 compared to CTRL from Day 14 to Day 30.](image-url)
BET Bromodomain inhibitor OTX015

Inhibition of the activity of important transcription factors

- MYC
- E2F1
- NFKB

DLBCL cell lines

OTX015 affects important biologic processes

transcripts coding for histones, overlapping with those up-regulated by HDAC-inhibitors

MYC and E2F1 targets, genes involved in NFkB/TLR/JAK/STAT pathways

OTX015 in lymphomas

Down-regulation of MYC and MYC targets

OTX015 down-regulated MYC and MYC targets

0 1 µM 1 µM 1 µM 1 µM OTX015
0 0.5 1 2 4 hours
DMSO
100 nM
200 nM
500 nM
DMSO
100 nM
200 nM
500 nM

SU-DHL-2

DoHH2

MYC
CAD
NUC

0 1 µM 1 µM 1 µM 1 µM OTX015 minutes
0 30 60 120 240

SU-DHL-2

MYC
CAD
NUC

0 1 µM 1 µM 1 µM 1 µM OTX015 minutes
0 30 60 120 240

OTX015 in lymphomas

MYC down-regulation is reversible

OTX015 in lymphomas

Down-regulation of TLR/JAK/STAT/NFκB
OTX015 in lymphomas

Down-regulation of active NFKB

p50 (NFKB1)

DMSO, 24hr

OTX015 500nM, 24hr

SU-DHL-2

OTX015 in lymphomas

Down-regulation of active JAK/STAT3

OTX015 down-regulates JAK activation

SU-DHL-2

pSTAT3 (Tyr705)

NT 8h
OTX 500nM 8h
NT 24h
OTX 500nM 24h

DMSO, 24hr

OTX015 500nM, 24hr

OTX015 in lymphomas

Assessment of combinations

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Category</th>
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<tbody>
<tr>
<td>Bendamustine</td>
<td>chemotherapy</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>chemotherapy</td>
</tr>
<tr>
<td>Bortezomib</td>
<td>Proteasome inhibitor</td>
</tr>
<tr>
<td>Ibrutinib</td>
<td>BTK inhibitor</td>
</tr>
<tr>
<td>Lenalidomide</td>
<td>Immunomodulant</td>
</tr>
<tr>
<td>Rituximab</td>
<td>anti-CD20 moAb</td>
</tr>
<tr>
<td>Vorinostat</td>
<td>HDAC inhibitor</td>
</tr>
<tr>
<td>Romidepsin</td>
<td>HDAC inhibitor</td>
</tr>
<tr>
<td>Decitabine</td>
<td>demethylating</td>
</tr>
</tbody>
</table>

three GCB-DLBCL
two ABC-DLBCL

OTX015 in lymphomas

DLBCL: synergism with several anti-cancer agents

Best:
- Everolimus
- Ibrutinib
- Rituximab
- Vorinostat

OTX015 synergises with different compounds

**HDAC-i**

**anti-CD20**

**mTOR-i**

**BTK-i**

ABC-DLBCL SU-DHL-2 xenograft

Bet Bromodomain inhibitors in lymphomas

Any biomarkers?
OTX015 in lymphomas

Apoptosis limited to a genetically defined subgroup of DLBCL

ABC-DLBCL
mut MYD88
mut in CD79B or mut CARD11
wt TP53

Mutations in MYD88 and for components of the BCR signalling: significantly associated with apoptosis (P = 0.027)

MYD88, CD79B and CARD11 act on the signalling

OTX015 in lymphomas

Complete responses in the Phase I

Figure 4: Waterfall plot showing anti-tumour activity in evaluable DLBCL patients (N=17) as maximal percent tumour... negative PET scan. Five patients were non-evaluable for variation relative to baseline (missing baseline or early PD).

OTX015 in lymphomas

Complete responses in the Phase I

Conclusions

• Anti-proliferative activity in mature B-cell lymphoid tumor cell lines
• Mainly cytostatic activity, but apoptosis in genetically defined subgroups
• Down-regulation of MYC and MYC targets target genes
• Down-regulation of MYD88, JAK/STAT3 and NFKB pathways
• Synergism with different agents
• Signs of clinical activity
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14th International Conference on Malignant Lymphoma

Palazzo dei Congressi
Lugano (Switzerland)
www.lymphcon.ch

SAVE THE DATE: June 14-17, 2017