THE TOXICOLOGIC PATHOLOGY OF COMMON KINASE TARGETS

IFSTP, IATP & STP-I
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Timothy LaBranche, DVM, PhD, DACVP
Disclaimer

- This talk is only intended to be an academic review of published toxicologic pathology data for educational purposes

- All assertions and opinions from this talk are solely those of the speaker and are not to be misconstrued as to represent the official position of their employer or any other sponsor referred to herein

- The audience is referred to the official drug label of the pharmaceutical for all usage and precaution information
Protein Kinases

- A type of enzyme which modifies other proteins by adding phosphate groups (phosphorylation)

- One of the largest and most functionally-diverse gene families
  1. Serine-threonine kinases (i.e. MAPKs, PKA, PKC)
  2. Tyrosine kinases (~15%):
     a) Receptor (i.e. EGFR, FGFR, PDGFR, KIT)
     b) Non-receptor (i.e. JAK, SYK)

- Regulate the activity, localization, and function of many proteins & cellular processes in a variety of organ systems (i.e. drive vascular growth, bone marrow development, immune function)

Manning G et al 2002 Science 298:1912-1934
Ghoreschi K et al 2009 Nat Immunol 10(4):356-360
Protein Kinases

- 518 human protein kinases (~2% of human genes). Most belong to a single superfamily, with related catalytic domain sequences.

- Can be clustered into groups, families and sub-families based on sequence similarity and biochemical function.

- Kinase dendrogram illustrates sequence similarity between catalytic domains:
  - Seven major groups:

<table>
<thead>
<tr>
<th>Kinase</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGC</td>
<td>Containing PKA, PKG, PKC families</td>
</tr>
<tr>
<td>CAMK</td>
<td>Calcium/calmodulin-dependent protein kinase</td>
</tr>
<tr>
<td>CK1</td>
<td>Casein kinase 1</td>
</tr>
<tr>
<td>CMGC</td>
<td>Containing CDK, MAPK, GSK3, CLK families</td>
</tr>
<tr>
<td>STE</td>
<td>Homologs of yeast Sterile 7, Sterile 11, Sterile 20 kinases</td>
</tr>
<tr>
<td>TK</td>
<td>Tyrosine kinase</td>
</tr>
<tr>
<td>TKL</td>
<td>Tyrosine kinase-like</td>
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</tbody>
</table>
Kinase Inhibitors: A Story of Tepid Success

- Kinase inhibitors have been developed for 20+ years
  - Dozens have been approved in the US, most for use in cancer
  - One kinase inhibitor has been approved in the U.S. for non-oncology: tofacitinib (Xeljanz®), a JAK1/3 inhibitor approved for us in moderate to severe rheumatoid arthritis; Fasudil - ROCK inhibitor approved in Japan.

- We’ve only focused on a handful of the known kinases, including both wild-type and mutated proteins
  - Point mutations
  - Fusion mutations
  - “Resistance mutation”: a mutation that reactives a kinase, potentially rendering the current treatment ineffective

*Bamborough P, 2012* *Expert Opin Drug Discov;* 7(11):1053-1070
Kinase Inhibitors: A Story of Tepid Success

- Non-selective kinase inhibitors have been prevalent
  - Conservation of the ATP binding pocket among kinases
  - Partly by design (multiple targets to ↑ chance of efficacy)
  - Partly due to technical limitations (conserved binding sites, lack of kinome-wide profiling)

- Medicinal chemistry approaches to improve selectivity:
  - ATP-competitive inhibitors (take advantage of non-conserved residues)
  - Non-competitive/covalent inhibitors (if unique cysteine residue)
  - Allosteric inhibitors (target regions remote from ATP binding pocket, i.e. DFG-out)

Selectivity

Kinome selectivity of some FDA-approved inhibitors:

Size of the circle corresponds to the degree of binding

Multi-kinase inhibitors are heavily biased towards the VEGFR, PDGFR classes. Other kinase inhibitors show much less polypharmacology
Webinar Housekeeping Notes

• Webinar Broadcast is online-only (no phone) so must have audio/speakers on your computer. For technical problems e-mail ifstp-hq@verizon.net.

• At any time, please send all questions to moderator: KnKe@NovoNordisk.com

• Do not use Webex “Chat” or “Q&A” buttons Questions will only be addressed from the e-mail above.

• Slides and a recording of this presentation will be uploaded to IFSTP website www.ifstp.net in one week.
COMMON KINASE TARGETS & ASSOCIATED TOX
Common Kinase Targets & Associated Tox

1) EGFR
2) p38
3) VEGFR
4) PDGFR
5) FGFR
6) JAK2
7) JAK1/3
EGFR

- Normal skin growth:
  
  1. Basal keratinocytes (cytokeratin-14 positive) proliferate and detach from the basement membrane.
  
  2. Migrate through the epidermis while undergoing terminal differentiation and formation of the cornified layer.

  - Controlled by $Ca^{2+}$ and kinase signaling pathways.
EGFR

- EGFR inhibition → premature keratinocyte maturation → necrosis/apoptosis → rash, perifollicular inflammation, papulopustular eruption

- Up to 90% incidence, dose-dependent

- Investigative / screening approaches:
  a) Keratinocyte proliferation assay (³H-thymidine uptake)
  b) Reconstituted human epidermal (RHE) system
  c) Plucked hair follicles

  Rodents and monkeys are less sensitive; predictive tools limited to human cells or 3D cultures

A - acneiform papulopustular rash
B – neutrophilic folliculitis w/ rupture

Brodell et al 2013 J Cutan Pathol; 40:865-870
- Gut Associated Lymphoid Tissue (GALT / Peyer’s patch) necrosis & neutrophilic inflammation $\rightarrow$ ileal hemorrhage
- Species-specific tox effect in dogs:
  - B cell death
  - p38α MAPK signaling pathway (i.e. p38α, MK2)
- Does your compound inhibit a kinase upstream and/or downstream to p38α?
  - Tox species selection
p38 MAPK

GALT necrosis / hemorrhage has not been observed in humans
VEGFR

- Vascular endothelial growth factor receptor (VEGFR)

- VEGF binds to:
  - VEGFR-1 (Flt-1)
  - VEGFR-2 (KDR/Flk-1)

- VEGFR-2 mediates most of the cellular effects:
  - Endothelial cell mitogenesis, survival and microvascular permeability…
VEGFR and Hypertension

Two hypotheses on VEGF inhibitor-induced hypertension:

1. Endothelial nitric oxide synthase (eNOS) is dependent upon VEGFR
   - Reduced NO production $\rightarrow$ ET-1 pathway stimulated $\rightarrow$ vasoconstriction and subsequent hypertension

2. VEGF maintains capillary network integrity
   - Decreased VEGF signaling $\rightarrow$ rarefaction / reduction of the density of capillary beds $\rightarrow$ hypertension

de Jesus-Gonzalez et al Hypertension 2012; 60: 607-615
**VEGFR and Physeal Dysplasia**

- Increased size of zone of hypertrophic chondrocytes
- Decreased invasion by new blood vessels
- Reduced interdigitation of cartilage and calcified matrix

*Normal Growth Plate:*

Chondrocytes make FGF & VEGF to modify the cartilage matrix → allows for capillary invasion → chondrocyte apoptosis → osteoblasts enter → bone matrix deposition & mineralization

**References:**
- Ryan AM et al 1999 *Tox Pathol*; 27(1): 78-86
- Patyna et al *Toxicol Pathol* 2008; 36(7): 905-916
VEGFR and Teeth

1. Loss and disorganization of odontogenic cells (i.e. odontoblasts, ameloblasts) and associated vasculature

2. Deposition of poorly-mineralized dentin (reduced eosinophilia) and loss of fine parallel dentine tubules.
Corpus lutea development is dependent upon proliferation of BVs within the theca interna

Reduced ovarian weight, decreased number of corpus lutea has been observed in rats given VEGF inhibitors:

Sunitinib (Sutent®) induced a time & dose-related impairment of the capillary microvasculature in rats

Markedly distended endothelial cells (arrows) compress the erythrocyte (*) at the center of the capillary lumen. Perivascular dilatation (a) is also evident. These capillary lesions were followed by cortical cell necrosis. *Transmission Electron Micrograph (TEM) 400X*

*Patyna et al Toxicol Pathol* 2008; 36(7): 905-916
VEGFR and other vascular tissues

Axitinib (Inlyta®) ↓'s endothelial fenestrations in pancreatic islets & renal glomeruli

Fenestrated capillary regression:
- Endocrine glands
- Choroid plexus
- Kidney
- Small intestine

Surviving endothelium was unusually thick, had greatly reduced numbers of fenestrations and contained abundant caveolae with diaphragms.

Kamba T Br J Cancer 2007; 96:1788 – 1795
PDGFRβ

- Platelet-derived growth factor-beta (PDGFβ) receptors are expressed by pericytes of the vasculature & fibroblasts within the stroma of solid tumors.
- Decreased pericyte recruitment / pericyte loss → vessel fragility and hemorrhage.

Cystic hemorrhagic dilatation / degeneration of the corpus lutea.

Hall AP et al *Toxicol Pathol* 2016; 44(1):98-111
FGFR

Fibroblast growth factor receptor (FGFR)

- In the kidney, FGFR suppresses the biosynthesis of 1,25(OH)2D3
- FGFR inhibition $\Rightarrow$ increased 1,25(OH)2D3-mediated phosphate absorption from the gut $\Rightarrow$ hyperphosphatemia $\Rightarrow$ increased Ca x P product (in the presence of normo- or hypercalcemia) $\Rightarrow$ soft tissue mineralization

**Stomach, heart, kidneys, aorta:**

Three hormones signal through JAK2:
1. Erythropoietin (EPO): RBCs
2. Thrombopoietin (TPO): platelets

Potential effects of JAK2 inhibition:
- Reduced bone marrow cellularity
- Anemia
- Thrombocytopenia
- Neutropenia, Eosinopenia & Monocytopenia
Reduced T cell populations, leading to opportunistic infections:

- **Rats** – rare, when colonies maintained under SPF conditions
- **NHPs** - bacterial diarrhea (i.e. *Campylobacter, Shigella, Yersinia, etc.*); polyoma virus infection (progressive multifocal leukoencephalopathy - PML); lymphoid hyperplasia, lymphoma
- **Dogs** - *Demodex canis*, interdigital pododermatitis, *Malassezia pachydermatitis*, canine papillomavirus
- **Humans** - recrudescent viral infections (*Herpes zoster* and shingles, Epstein-Barr virus and lymphoma); opportunistic bacterial (*Mycoplasma tuberculosis*) and fungal (oral candidiasis) infections

Haley PJ 2012 *Tox Pathol*; 40:261-266

FDA CDER Summary Review for tofacitinib:
http://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/203214Orig1s000SumR.pdf
Summary

- Using structural knowledge to guide rational drug design and discovery, selective inhibition of protein kinases is possible...and toxicity can be minimized

- Which selectivity profile do you really need?

- What off-target kinases activities are acceptably safe?
Summary

- As an industry, we benefit from shared knowledge

- How can we share?
  a) Seminars like this (Thank You to: IFSTP, IATP & STP-I)
  b) “Something like a ClinicalTrials.gov for preclinical work”?
     (https://www.statnews.com/2016/06/10/califf-database-preclinical-trials/)
  c) A ‘Kinase Safety Database’?
The unique cysteine in the FGFR4 hinge region (Cys552, hinge-1 residue) provides an opportunity for selective inhibition through covalent modification.

This concludes the webinar presentation. Attendees may submit their questions for the speaker to the moderator:

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Thank you for attending.