

Nibs and Mabs: New Approaches to fighting cancer

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What are kinases?

Kinases are proteins that have the ability to phosphorylate other proteins

Activation initially involves phosphorylation of tyrosine or serine/threonine; requires ATP

This phosphorylation permits binding of other cytoplasmic molecules to the receptor, leading to further phosphorylation and downstream signaling

Kinases can be on the cell surface, in the cytoplasm, and in the nucleus

Kinase Signaling

Stimulation of kinases initiates an ordered sequence of biochemical reactions within a cell, resulting in: Gene activation/inhibition, Proliferation, Survival, Migration

Receptor Tyrosine Kinases and Angiogenesis

Kinases and Cancer

Kinases are often dysregulated in cancer

Mechanisms of dysregulation include: overexpression, mutation, chromosomal, translocation, autocrine loop of activation

These contribute to growth factor independent signaling that contributes to uncontrolled cell growth, survival, and motility

RTKs are often implicated in this dysregulation: they stimulate the growth of tumor cells and are important mediators of tumor- stromal cell and tumor-endothelial cell interactions involved in angiogenesis

RTK Dysregulation Is Associated With Many Human Cancers (table insert)

KIT Dysfunction Plays a Role in the Proliferation of Canine and Possibly Feline MCT

KIT is critical for the development and growth of canine mast cells

Expressed on mast cells and activated by stem cell factor (SCF)

Prevalence of KIT mutations is approximately 25% in grade 2/3 canine MCTs

Internal tandem duplications (ITDs) in exons 11 and 12 (juxtamembrane domain) lead to constitutive phosphorylation of KIT in the absence of SCF binding
KIT ITD mutations are associated with an increased rate of recurrent disease and mortality in dogs with MCT
Exon 8 and 9 mutations have been identified and are constitutively activating

Strategies to Inhibit Tyrosine Kinases

Most TKIs work by blocking the ability of the kinase to bind ATP (i.e., competitive inhibitor), as this is necessary for donating the phosphate group that phosphorylates the TK as well as downstream targets; inhibitors are often called small molecule inhibitors
RTKs can also be inhibited through the use of monoclonal antibodies that bind the extracellular domain of the target; this does not necessarily alter the phosphorylation status of the RTK, but may lead to receptor endocytosis and downregulation or immune mediated tumor cell destruction (complement/ADCC)

Small Molecule Inhibitors

Small molecules designed to target a specific protein, often at ATP binding pocket
Block the function of that protein and disrupt cellular processes/signaling
Usually given orally, sometimes intravenously and work at low micromolar or nanomolar concentrations
Effectiveness often dependent on the reliance of tumor cells on that pathway
Toxicities result from blocking pathways in normal cells
Inhibitors can be very specific or may have multiple off-target effects

Therapeutic Efficacy

Dependent on extent/duration of target inhibition
Tumor cells need to rely on the particular pathway being targeted
Unlike chemotherapeutics, activity may not be completely tied to maximum tolerated dose (MTD)

- effective target inhibition may occur below the MTD

Unlike chemotherapeutics that often irreversibly damage DNA, target inhibition is often reversible

- pathway inhibition usually relieved following metabolism of therapeutic

Resistance usually driven by mutation in target binding site that prevents inhibitor from binding, or development of alternative signaling pathways

Small-Molecule Inhibitors Have Proven Effective in Treating Human Cancers (table insert)

Small-Molecule Inhibitors and Human Cancers (table insert)

Tyrosine Kinase Inhibitors in Veterinary Medicine

Limited use in veterinary medical oncology - lack of well-defined targets for specific cancers, little data regarding safety and toxicity of many agents, some therapeutics cannot be used (i.e., humanized monoclonal antibodies), often cost prohibitive

Canine/feline cancers for which targets have been identified - mast cell tumors, osteosarcoma, GIST, sarcomas

Small molecule inhibitors that have been evaluated in dogs/cats - Gleevec, Kinavet, Palladia

Gleevec (imatinib mesylate)

Oral small molecule inhibitor

Inhibits binding of ATP

Active against Bcr-Abl, Kit, and PDGFR

Originally designed to inhibit the bcr-abl fusion protein found in human patients with CML

- approximately 90% remission rate

Inhibits Kit signaling - over 50% remission rate for human gastrointestinal stromal tumors which possess activating mutations in Kit similar to those mutations in canine MCTs

Gleevec Use in Dogs and Cats

Few publications; hepatotoxicity can be limiting and has been reported anecdotally

Isotani et al, 2008: response to therapy in 10/21 dogs treated with Gleevec; response rate was 100% in

dogs with MCTs possessing a Kit ITD (n=5, 1 CR, 4 PR)

Marconato et al, 2008: response to therapy in 3 dogs with systemic mast cell disease treated with Gleevec.

Isotani et al, 2008: response to therapy in a cat with systemic mastocytosis and exon 8 Kit mutation

Dosing is somewhat empirical (4-10 mg/kg) and often based on available tablet sizes (100 mg/400 mg)

Isotani et al, 2008: Response to therapy in a cat with systemic mastocytosis and exon 8 Kit mutation

Kinavet (Masitinib mesylate)

Small molecule RTK inhibitor: 2-aminothiazole, AB Science

Competitive inhibitor of ATP binding; prevent phosphorylation on tyrosine of Kit and others

Effective against : Kit, PDGFR, (FGFR3?)

IC 50 for Kit inhibition: 0.005-0.030 mM

Kinavet: Clinical Field Study

Recurrent or non-resectable Grade II or III MCTs

May have never received treatment

No evidence of lymph node or systemic disease

Baseline target lesion of at least 1 cm

Design - Dogs were randomized to receive either masitinib or placebo in a 4:1 ratio, evaluation at weeks 1, 2, 4, 6, and 2 mth, 3 mth, 4 mth, 5 mth, and 6 mth following study entry, response assessment at 4 mths, confirm at 6 mths, no placebo escape, dogs allowed to continue on drug after study completion

if doing well

Kinavet: Summary of Responses

Masitinib increased overall time to progression (TTP) compared with placebo from 75 to 118 days (n=202, P = 0.038).

This effect was more pronounced when masitinib was used as first-line therapy: increase in the median TTP from 75 to 253 days (P = 0.001)

TTP was also increased for dogs receiving masitinib whether the tumors expressed mutant (83d vs not reached: P = 0.009) or wild-type Kit (66d vs 253d: P = 0.008).

There was no statistically significant difference in ORR between placebo (14.7%) and masitinib (15.6%)

Kinavet: Summary of Responses

“Rate of best response” was very high in both placebo and masitinib treated dogs

Placebo: CR=21% CR+PR=36%

Masitinib: CR=26% CR+PR=55%

The spontaneous (placebo) CR rate reported in this study is extremely high, and has not been reported in other MCT studies

Palladia (toceranib phosphate)

Small molecule RTK inhibitors developed by SUGEN, Inc.: SU5416, SU6668, SU11248, SU11654 (*Palladia*), indolinone cores

Competitive inhibitor of ATP binding; prevents phosphorylation on tyrosine of associated RTK
Effective against members of the “split kinase” family including: VEGF-R, PDGF-R, Flt-3, Kit
Both direct anti-tumor and direct anti-angiogenic activity

Palladia Phase I Study

57 dogs entered into study

Established maximum tolerated dose: 3.25 mg/kg EOD

Investigated pharmacokinetics in dogs with cancer and established appropriate dosing regimen

6 complete responses, 10 partial responses, 15 dogs with stable disease for >10 wks

16 of 57 with response to therapy: 28%

mast cell tumors

sarcomas, carcinomas, myeloma, melanoma

31 of 57 considered to have evidence of biological activity: 54%

Grade II MCT with Kit Mutation (photos shown)

Clinical Summary: Mast Cell Tumors

Kit Mutation Positive

4 CR, 5 PR, 1 SD

Biological activity 10/11=91%

Kit Mutation Negative

1 CR, 1 PR, 1 SD

Biological activity 3/11=27%

- Dogs with ITDs were more likely to respond to Palladia therapy: $p=0.003$
- Dogs with no evidence of lymph node involvement were more likely to respond to Palladia therapy: $p=0.03$

Katie: Metastatic Mammary Carcinoma (photo shown)

Fawn: Multiple Myeloma (graph insert)

Palladia: Clinical Field Study

Recurrent Grade II or III MCTs: lymph node metastasis eligible, no evidence of systemic disease, baseline target lesion of at least 2cm, response assessment at 6 weeks, option for escape to active drug if on placebo

Design: dogs were randomized to receive either Palladia or placebo in a 4:3 ratio, weekly evaluation for the first 6 weeks, response assessment at weeks 3 and 6, if progressive disease at week 3 or 6, unblind and eligible for active drug if on placebo, blinded phase ends at 6 weeks, with continuation in open-label phase; rechecks every 3-6 weeks in open-label phase

Study Design (chart shown)

Palladia: Summary of Objective Response (graph shown)

Palladia: Summary of Biologic Response

- Among dogs receiving Palladia during the blinded phase, the objective response rate for dogs with KIT ITD mutation was 60.0% and 31.3% for dogs without the mutation ($P=0.0099$)
- During the blinded plus the open label phase, the objective response rate in dogs with KIT ITD mutation was 69% compared to 36.8% in dogs that did not possess the mutation

Treatment of Mast Cell Tumors

Reported single agent response rates in the setting of gross disease:

- Vinblastine (n=100):	12-27%
- Lomustine (n=19):	42%
- Palladia (n=145):	42.8%

Reported multi-agent response rates in the setting of gross disease:

- Vinblastine/Prednisone (n=15):	47%
- Vinblastine/Cytosar/Prednisone (n=11)	63%
- Vincristine/Cytosar/Hydroxyurea/Pred (n=17)	59%

Given the significant activity of Palladia as a single agent, it is possible that, similar to the case with other chemotherapeutics, Palladia will have even greater activity when administered in combination with prednisone

My Thoughts on the Clinical Utility of Gleevec and Kinavet

Both drugs inhibit Kit and PDGFR

Biologic activity would be expected in tumor types where Kit and PDGFR may be dysregulated

Canine and feline mast cell tumors

Tumors with negative prognostic indicators, high grade tumors, recurrent tumors, non-resectable/multiple tumors, kit mutation positive tumors

Canine GISTs

Kit positive

My Thoughts on the Clinical Utility of Palladia

Biologic activity would be expected in tumor types where Kit, PDGFR, VEGFR may be dysregulated or where an anti-angiogenic effect was desired

MCT with gross disease

Vinblastine/Pred-based therapy, followed by Palladia/pred

Grade 3 tumors, Grade 2 tumors requiring medical treatment (i.e., negative prognostic indicators..LN+, rapid growth, recurrence, etc), multiple MCTs/large non-resectable tumors,

KIT mutation positive tumors

MCT with microscopic disease

Vinblastine/pred-based therapy, followed by Palladia/Pred if Grade 3 tumor or if Grade 2 tumor with negative prognostic indicators

My Thoughts on the Clinical Utility of Palladia: Other Tumors

1. Gastrointestinal Stromal Tumors

Similar to GIST in people, dog GISTs often carry Kit mutations like those found in MCTs, human GIST respond well to Kit inhibitors

2. Soft Tissue Sarcomas

Responses noted in Phase I study, mechanism unclear (PDGFR/anti-angiogenic), currently no effective chemotherapy for metastatic soft tissue sarcomas

3. Mammary Carcinomas

Responses noted in Phase I study, mechanism unclear (PDGFR or Kit/anti-angiogenic), currently no effective chemotherapy for metastatic mammary tumors

4. Multiple Myeloma

Response noted in Phase I study, mechanism unclear (VEGFR, Kit/anti-angiogenic), currently no available effective therapy for relapsed MM

Side Effects of Kinase Inhibitors

All kinase inhibitors have toxicities

The spectrum of toxicities is often dictated by the array of receptor/target inhibition

- In general, the more receptors inhibited, the more toxicities observed

Toxicities observed are often similar to those that occur with other systemic therapies such as chemotherapy

In most instances, toxicities can be prevented or readily managed with appropriate supportive care or dose modulation/schedule modulation

Life-threatening toxicities are rare, although early recognition of potential problems is critical

Side Effects of Kinase Inhibitors

Spectrum and severity of toxicities are likely influenced by several factors including:

- stage of disease: dogs with more advanced disease generally have a lower performance score that could potentially influence toxicities; this has been shown to be the case in humans treated with multi-targeted therapies

- type of cancer: dogs with macroscopic MCTs are known to have high circulating levels of histamine that can predispose to GI ulceration and other GI toxicities; these could be compounded by Palladia therapy

- pre-existing conditions: liver disease, renal disease, cardiac disease can all influence performance scores, and these may also impact drug metabolism/elimination thereby compounding toxicities

- concomitant meds: certain drugs may exacerbate GI toxicity or impair drug metabolism

Side Effects of Kinase Inhibitors

Anorexia, lethargy, diarrhea, GI bleeding, vomiting

Agent specific toxicities: neutropenia (Palladia), muscle cramping (Palladia), hypoalbuminemia (Kinavet), protein losing nephropathy (Kinavet), hepatotoxicity (Gleevec)

Prevention/Management of Side Effects

Administer with food/a meal

H2 blockers or proton pump inhibitors may help prevent GI irritation/ulceration, particularly in MCT patients (famotidine, omeprazole, sucralfate, misoprostol)

Anti nausea agents may help with anorexia (metoclopramide, ondansetron, Cerenia)

Medications that treat or prevent diarrhea (Peptobismol, loperamide (Imodium), metronidazole)

If clinical signs do not readily resolve, consider treatment break with alteration of dose and/or schedule

Summary of Adverse Drug Events (AEs) 10/1/09 – 2/28/10

81 AEs Reported

77 Adverse responses with 14 mortalities: majority (62/77) reported in mast cell tumor (MCT) patients, 15/77 reported in other tumor types

3 Lack of efficacy

1 Human exposure

Clinical Signs Reported in Non-Mortality Cases

10/1/09 – 2/28/10

Emesis (40%), Diarrhea/hemorrhagic diarrhea (38%), gastroenteritis/abdominal pain (36%), lameness/joint pain/local pain/musculoskeletal disorder (25%), lethargy (22%), anorexia (18%), alopecia (11%), GI tract hemorrhage (11%), skin lesions/delayed wound healing/dermatitis/abscess (9%), pigmentation disorder (9%), muscle tremor (7%), pancreatitis (7%), ataxia (4%), hepatomegaly (4%), convulsion, cataract, tachycardia, doughy abdomen (2%)

AEs occurring in >10% of PALLADIA-treated dogs during blinded phase of clinical trial

Diarrhea (46.0%), anorexia (includes decreased appetite) (39.1%), lethargy (35.6%), vomiting (32.2%), lameness (17.2%), weight loss (14.9%), musculoskeletal disorder (11.5%), blood in stool/GI bleed/hemorrhagic diarrhea (12.6%)

Laboratory Abnormalities Reported in Non-Mortality Cases, 10/1/09 – 2/28/10

Neutropenia/leukopenia (29%), Anemia (11%), Thrombocytopenia (7%), Hepatopathy (4%), Elevated BUN (4%), Neutrophilia (2%)

Laboratory AEs occurring in >10% of PALLADIA-treated dogs during blinded phase of clinical trial

Neutropenia (46.0%), Thrombocytopenia (24.1%), ALT (24.1%), Albumin (12.6%)

Mortality Adverse Event Reports (n=14), 10/1/09 – 2/28/10

79% (11/14) were in poor prior condition

MCT disease had been diagnosed for an average of 4.6 months (range = 1 week to 12 months) prior to initiation of PALLADIA treatment

Average treatment duration was 11 doses, EOD (range = 5 to 51 doses)

11/14 cases (79%) reported using a dose reduction and/or drug holiday to manage the adverse reactions

8/14 cases were most likely euthanized or died due to progression of disease

6/14 cases were more difficult to determine if the direct chemotherapeutic side effects or the complications associated with MCT necrosis and subsequent histamine release was the cause of the poor outcomes

Non-Mortality Adverse Event Reports (n=63), 10/1/09 – 2/28/10

Majority (52/63) had MCT; 11/63 non-MCT, Average dose was 3 mg/kg, Average length of treatment before a reaction was observed was 4 weeks (range 1 dose to 22 weeks), 30% described PALLADIA dose reductions, treatment breaks and/or dose schedule modifications that were instituted to manage AEs, Palladia was discontinued in 20/63 (32%) of these cases due to AEs (75% of those 20 cases did not report any attempts at dose reductions or drug holidays), Majority were monitored closely by veterinarians weekly or q 2 weeks (Illustrates that early detection and medical intervention are critical), Most frequently used concomitant medications: GI protectants, Prednisolone on alternating days with PALLADIA, Maropitant, Metronidazole

Palladia in non-MCT patients

Phase I study indicated potential biologic activity in tumor types beyond MCT: Metastatic carcinomas and sarcoma (STS, OSA), Melanoma, Myeloma

Several dogs experienced SD for extended periods (6 months) and a few underwent partial responses to therapy

Multi-targeted RTKs similar to Palladia (Sutant/Sorafenib) have shown activity in several different tumor types in people.

Palladia in non-MCT patients: Phase I study (table shown)

Palladia in non-MCT patients

Tumor Types: Anal gland adenocarcinoma n = 16, Thyroid carcinoma n = 9, Squamous cell carcinoma

n = 5, Osteosarcoma n = 4, Hemangiopericytoma n = 2, GIST n = 2, Hemangiosarcoma n = 2, Primary lung adenocarcinoma n = 2, Histiocytic disease, melanoma, nasal adenocarcinoma, TCC, gastric CA, neuroendocrine CA, nephroblastoma, renal cell CA, vaginal CA

Palladia in non-MCT patients

Time on Palladia: Median: 18 weeks (126 days, mean 18.1 weeks), Range: 4-56 weeks

Overall response to therapy: SD n = 29, PR n = 16, MR n = 3, CR n = 3

Palladia in non-MCT patients: Anal gland ACA

Response to therapy: SD: n = 10, PR: n = 5, MR: n = 1

Anal Sac Adenocarcinoma (radiographs shown)

Palladia 3.25 mg/kg EOD, then 2.7 mg/kg EOD; on therapy for 5+ months

Anal Sac Adenocarcinoma (radiographs shown)

Palladia 2.72 mg/kg MWF for 3+ months

Goal of Targeted Therapy

To identify abnormal protein/pathway common to certain cancers and to develop therapies that specifically target this pathway

Approaches include: monoclonal antibodies, small molecules, anti-angiogenic agents, anti-sense approaches, gene therapy, tumor vaccines

Targeted Therapy: The Human Experience

Several successes in the human arena, most have involved the use of monoclonal antibodies or small molecule inhibitors, in some instances, the targeted therapies have become part of standard of care, although this process often takes years. Herceptin for breast cancer, Rituximab for B cell LSA, Gleevec for CML/GIST

Targeted Therapy: The Human Experience

The effectiveness of targeted therapies is generally dependant on the reliance of tumor cells on the protein/pathway affected by the therapy - aberrant expression of a receptor/pathway does not mean the tumor cell is necessarily relying on that receptor/pathway for growth/survival

Inhibitors can be very specific, as in the case of monoclonal antibodies, or have multiple targets as

occurs with several small molecule inhibitors

Toxicities result from effects of target inhibition on normal cell types

Small Molecule Inhibitors

Most small molecule inhibitors work by blocking the ability of their target to engage in functional activity.

For several targets this entails preventing the binding of ATP, as this is critical for donating phosphate groups necessary for the initiation/maintenance of signaling.

In some instances, small molecule inhibitors act to prevent protein-protein interactions that are necessary for signaling

Usually given orally, sometimes intravenously and work at low micromolar or nanomolar concentrations

Therapeutic Efficacy

Dependent on extent/duration of target inhibition

Tumor cells need to rely on the particular pathway being targeted

Unlike chemotherapeutics, activity may not be completely tied to maximum tolerated dose (MTD)

- effective target inhibition may occur below the MTD

Unlike chemotherapeutics that often irreversibly damage DNA, target inhibition is usually reversible

- pathway inhibition usually relieved following metabolism of therapeutic

Resistance typically driven by mutation in target binding site that prevents inhibitor from binding, or development of alternative signaling pathways

Resistance to targeted therapeutics

Resistance to targeted therapeutics is a common occurrence, especially when used in the gross disease setting, and when the inhibitor is used as a monotherapy

Resistance may occur through multiple mechanisms - development of mutations in the target that limit or preclude drug binding, gene duplication overwhelming the inhibitor, development of additional dysregulation/mutation/pathways that circumvent the inhibitor

Evidence suggests that in many cases, resistance is present in small numbers of tumor cells at the initiation of treatment

This supports the notion that use of targeted therapies in the microscopic disease setting is likely to provide more durable tumor control

Targeted therapies in veterinary medicine

Limited use in veterinary medical oncology - lack of well-defined targets for specific cancers, little data regarding safety and toxicity of many agents, some therapeutics cannot be used (i.e., humanized monoclonal antibodies), often cost prohibitive

Canine/feline cancers for which targets have been identified - mast cell tumors, osteosarcoma, GIST, sarcomas

Small molecule inhibitors that have been evaluated in dogs/cats - Gleevec, Kinavet, Palladia

Target profiles of imatinib, masitinib, toceranib (graph shown)

Response to Gleevec (photos shown)

Current status of Kit inhibitors and MCTs

The development path for Kit inhibitors in veterinary medicine mirrored that in human medicine -Analysis of target specificity, proof of target modulation in vitro required, demonstration of activity in mouse models, from identification of an inhibitor to approval takes years, It is clear that Kit inhibitors have biologic activity against MCTs although challenges exist with respect to the exact role these inhibitors should play in treating mast cell disease, should treatment be based on the presence of Kit mutation?, how should Kit inhibitors be combined with standard therapies?, what should be the duration of treatment?, macroscopic vs microscopic disease setting?

Role of multi-targeted inhibitors beyond MCTs

Palladia inhibits VEGFR in addition to PDGFR and thus may be useful in the anti-angiogenic setting used in metronomic protocols

Evidence now suggests that VEGF/VEGFR inhibitors when combined with low dose cytotoxics have broad activity in the metronomic setting

Recent human clinical trials have shown biologic activity of metronomic protocols combining Avastin (mAb directed against VEGF) with cytotoxics (cytoxan plus capecitabine or methotrexate)

-63% of patients in one study with metastatic breast cancer had PR (31.8%)/SD (31.8%) >24 wks

-68% of patients in one study with metastatic breast cancer had CR (2%), PR (46%), or SD (41%)

Preliminary data suggests that Palladia can be safely administered with cytoxan and certain NSAIDs; biologic activity noted in Anal Sac ADC, Thyroid CA, SCC, OSA

Challenges of metronomic anti-angiogenic therapy

While both cytotoxic agents and anti-angiogenic agents appear to be critical for effective metronomic therapy, there is little data regarding the class of cytotoxic that is most appropriate.

Dosing regimens for cytotoxics in metronomic protocols are generally empirically derived and not based on clear modulation of circulating endothelial precursors

Efficacy in the microscopic disease setting is generally unknown and for the most part untested

There are currently no established biomarkers to monitor the potential efficacy of metronomic protocols that incorporate VEGF/VEGFR inhibition - Plasma VEGF, Circulating endothelial precursors, Soluble VEGFR

Targeted therapies: Future directions

Several new targets are under investigation in the human clinical arena

Many of these are now being explored in veterinary medicine – mTOR, STAT3, HSP90, HDACi, Her2/Neu, CD20

Targeted therapies: HDAC inhibitors

Global DNA hypermethylation and histone hypoacetylation resulting in suppression of gene expression is a hallmark for many cancers

HDACi treatment results in hyperacetylation of H3 and H4, which induces re-expression of previously silenced genes in cancer cells, such as P21 and TRAIL

Other non-histone targets, such as HSP90, STAT3 and tubulin are also acetylated following HDACi treatment, altering their function.

These changes presumably restore the ability of cells to undergo cell cycle arrest and apoptosis

SAHA in the treatment of MCTs (photos shown)

Summary

The number of targeted agents in active human clinical trials has increased dramatically over the past 5 years

Despite this, few agents have demonstrated substantial single-agent activity

Significant challenges remain regarding how best to use these agents, particularly with respect to identifying those individuals most likely to respond to therapy

In the human arena, targeted therapies have only recently been used in the microscopic disease setting. Studies are ongoing regarding how best to combine these agents with standard of care (i.e., RT and chemo)

New Clinical Trial

- Partially funded!, Splenic hemangiosarcoma, 5 doses of doxorubicin, Toceranib “maintenance”