Jeffrey Dach MD
Bioidentical Hormones Natural Thyroid

Itraconazole Anti-Cancer Anti-Fungal Drug

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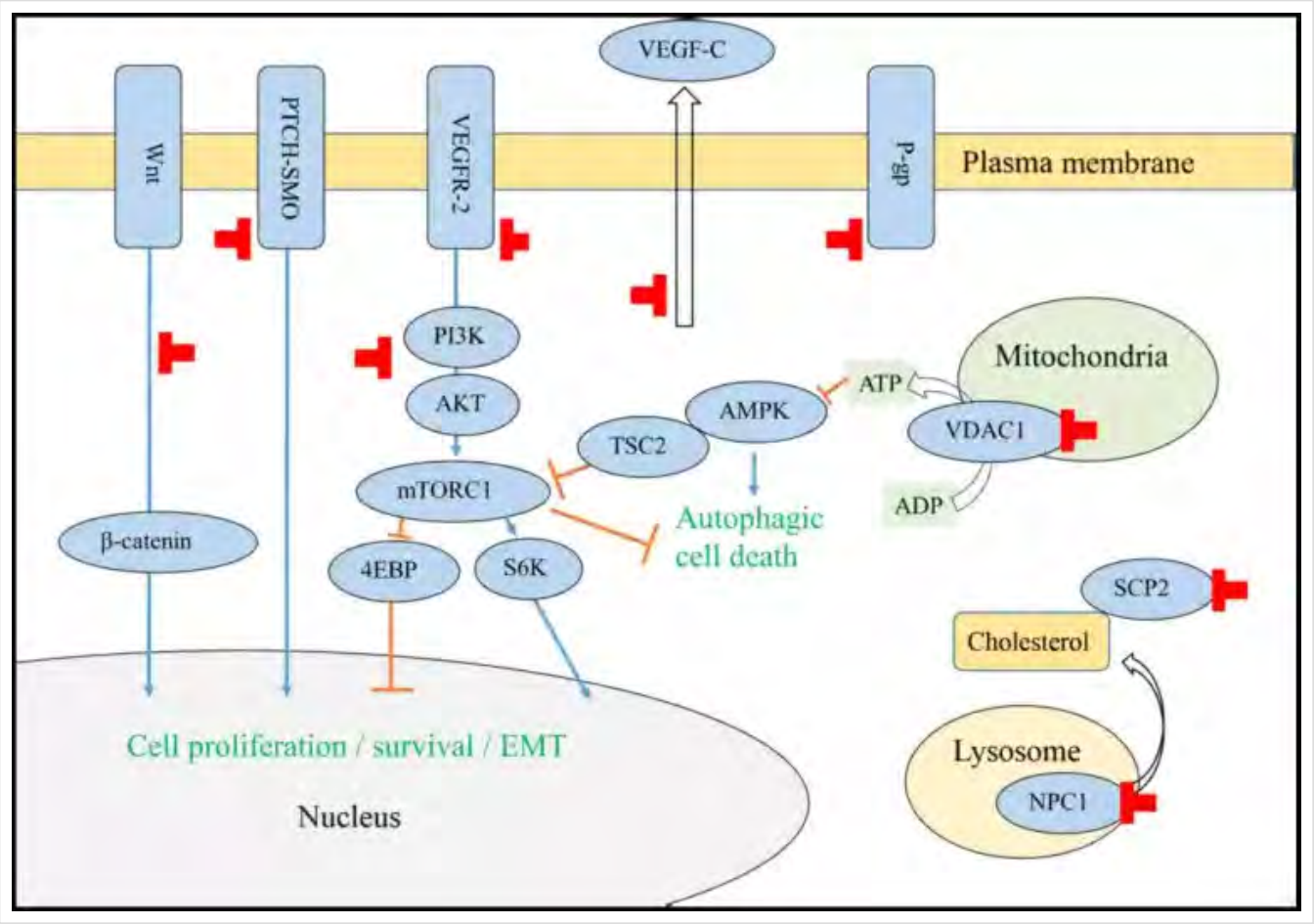
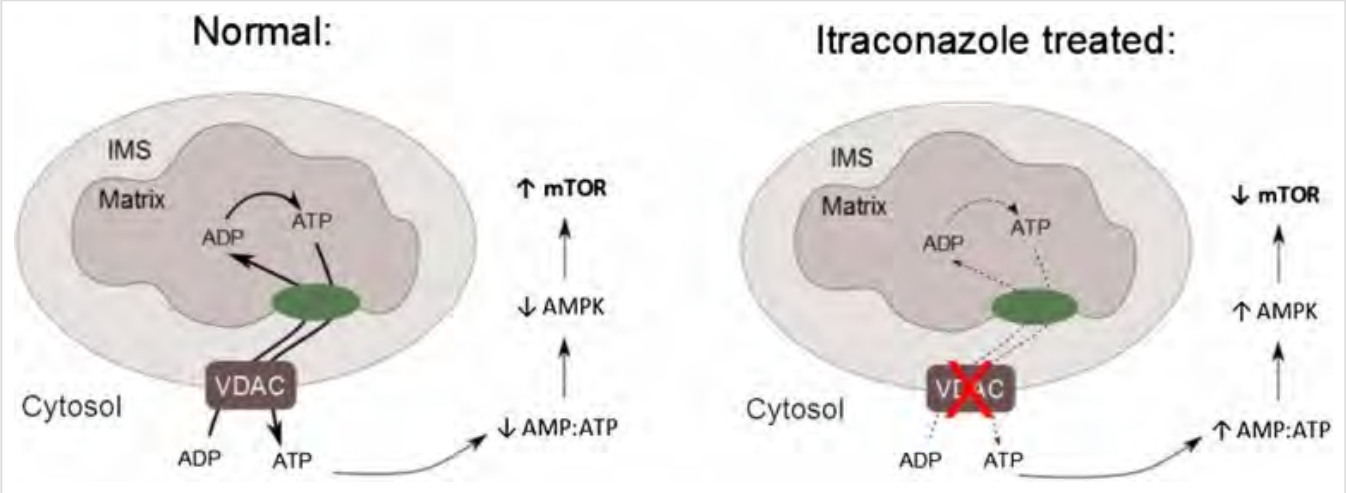


Itraconazole Anti-Fungal Drug Repurposed as Anti-cancer Drug
by Jeffrey Dach MD

Itraconazole, also known as Sporonox, is a common anti-fungal agent that was developed in the 1980s. It has been in clinical use for 30 years with an established safety record.(1-6) Multiple phase 2 clinical trials investigating itraconazole for non-small cell lung cancer, prostate cancer, and basal cell carcinoma have been completed, showing increase in the progression-free and overall survival. (1-6)

Retrospective studies of patients with ovarian cancer and recurrent triple-negative breast cancer taking itraconazole revealed significant increases in overall survival thought to be due to antiangiogenic effects of itraconazole. Other angiogenesis-dependent diseases such as macular degeneration and diabetic retinopathy may benefit from itraconazole ,as well.(1-6)

Header Image Fig 1 Courtesy of: Tsubamoto, Hiroshi, et al. "Repurposing itraconazole as an anticancer agent." Oncology Letters 14.2 (2017): 1240-1246. Figure 1. – Schematic representation of the anticancer activity of itraconazole. AKT, protein kinase B; AMPK, AMP-activated protein kinase; 4EBP, eukaryotic translation initiation factor 4E binding protein; EMT, epithelial-mesenchymal transition; mTORC1, mechanistic target of rapamycin complex 1; NPC1, Niemann-Pick C1 protein; P-gp, P-glycoprotein; PI3K, phosphoinositide 3-kinase; PTCH-SMO, transmembrane receptor protein patched-transmembrane protein Smoothened (SMO); S6K, ribosomal protein S6 kinase; TSC2, tuberous sclerosis complex 2; VDAC1, voltage-dependent anion-selective channel 1; VEGF, vascular endothelial growth factor; VEGFR-2, VEGF receptor 2.



Above image courtesy of Head, Sarah A., et al. "Antifungal drug itraconazole targets VDAC1 to modulate the AMPK/mTOR signaling axis in endothelial cells." Proceedings of the National Academy of Sciences 112.52 (2015): E7276-E7285. A model of VDAC1 inhibition mediating activation of AMPK and inhibition of mTOR. Under normal conditions, VDAC allows the passage of ADP/ATP into and out of the mitochondria, maintaining normal rates of ATP production and keeping basal AMPK activation low and mTOR activity high. Upon VDAC binding by itraconazole, mitochondrial ADP/ATP permeability is decreased, leading to a drop in ATP production, which causes AMPK activation and ultimately mTOR inhibition.

Itraconazole for Double Hit Lymphoma

Double hit lymphoma (DHL) is an extremely aggressive cell type refractory to conventional chemotherapy, and median survival is less than 1.5 years.(2) An in-vitro study by Dr. Juan J Gu in Blood 2016 using three Double Hit lymphoma cell lines with escalating doses of itraconazole showed Loss of ATP, Caspase 3/7 activation and loss of mitochondrial membrane potential.

*"Itraconazole caused G1 cell cycle arrest and decrease S phase in DHL. Moreover, itraconazole had a strong synergistic anti-tumor effect combined with BCL-2 inhibitor ABT199 (**Venetoclax**) , c-MYC inhibitor JQ1, bruton's kinase inhibitor **ibrutinib** and proteasome inhibitor (**bortezomib**)".(2)*

In Blood 2016, Dr Juan J Gu studied Chemotherapy resistance NHL (non-hogkins lymphoma) cell lines in vitro and found itraconazole enhances the efficacy of chemotherapy agents.(3) *"The disruption of HKII from mitochondria following itraconazole exposure may contribute to lower the mitochondrial membrane potential and enhance the chemotherapeutic efficacy. Our finding highlights itraconazole as a potential therapeutic agent in the treatment of B-cell malignancies, and strongly supports clinical translation of its use." (3)*

Repurposing Itraconazole as Anti-Cancer Agent

Dr. Hiroshi Tsubamoto wrote "Repurposing itraconazole as an anticancer agent." in Oncology Letters 2017.(4) He says:

Itraconazole, a common anti-fungal agent, has demonstrated potential anticancer activity, including:

1. reversing chemoresistance mediated by P-glycoprotein,
2. modulating the signal transduction pathways of: Hedgehog, mechanistic target of rapamycin (mTOR), and Wnt/β-catenin in cancer cells
3. inhibiting angiogenesis and lymphangiogenesis
4. interfering with cancer-stromal cell interactions.

Clinical trials using itraconazole monotherapy have shown clinical benefit in **prostate cancer and basal cell carcinoma,** and survival advantage of itraconazole combined with chemotherapy for **relapsed non-small cell lung, ovarian, triple negative breast, pancreatic and biliary tract cancer**.(4)

Binds to VDAC

Dr Sarah Head in a 2015 study in Proc Nat Acad Sci showed that itraconazole binds directly to the VDAC (Voltage Dependent Anion Channel) on the mitochodrial membrane and modulates the AMPK/mTOR signalling pathway, a regulator of angiogenesis. (5) Dr head's group synthesized a flourescent probe of itraconazole to identify the voltage-dependent anion channel 1 (VDAC1) as a "primary binding protein of itraconazole".(5) When itraconazole binds to VDAC1, this interferes with mitochondrial function, causing reduction in cellular energy level, which in turn triggers AMP-activated protein kinase (AMPK) which down-regulates mTOR activity leading to inhibition of endothelial cell proliferation (angiogeneisis).(5) Another effect discovered by Dr Head is cholesterol trafficking inhibition by itraconazole due to direct inhibition of the lysosomal protein NPC1. (6)

Cervical Cancer In Vitro

A 2017 Anticancer Res study using cervical cancer cells in vitro by Dr Ueda showed that itraconazole modulates Hedgehog, WNT/ß-catenin, as well as Akt Signalling, and Inhibits Proliferation of Cervical Cancer Cells. (7) They

found “8-fold down-regulation in the expression of GLI1, WNT4 and WNT10A among itraconazole-treated cervical cancer (CaSki) cells.(7) There was suppression in ß-catenin expression and Akt phosphorylation.(7)

Clinical Trials

There are many ongoing Clinical Trials using Itraconazole for gastric, pancreatic,esophageal, lung prostate , gynecologic, basal cell cancers.

Prostate Cancer Clinical Trial

A 2013 clinical trial was published by Dr Emmanuel Antonarakis in *The oncologist* using itraconazole in men with metastatic castration-resistant prostate cancer. (8) 46 men were randomized to receive either low dose , 200 mg per day or high dose, 600 mg per day of itraconazle. Progression free survival was determined by PSA. “The **PPFS rates at 24 weeks were 11.8% in the low-dose arm and 48.0% in the high-dose arm. The median PFS times were 11.9 weeks and 35.9 weeks”(8)** 50% of the men remained progression free for 6 months on the 600 mg per day itraconazole.(8)

Immune Suppression ?

Immuno-suppressive effects of itraconazole in-vitro were found to be artifacts when studied in-vivo.(9-12)

Potent Inhibitor of 5-LOX

In 1989, Dr K Jaschonek found that itraconazole a potent inhibitor at (low serum concentrations) of 5-lipoxygenase activity in human polymorphonuclear leukocytes (PMNL). (13)

Wnt, Cox-2 and 5-LOX

COX-2 inhibitors such as Celecoxib

In 2016 Dr Jessica Roos studied the regulation of tumorigenic Wnt signaling by cyclooxygenase-2, 5-lipoxygenase and their pharmacological inhibitors. (43) She reports that NSAIDSs like celecoxib suppress Wnt signaling by targeting the pro-inflammatory enzyme 5-lipoxygenase (5-LOX) . Inhibition of 5-LOX 5-lipoxygenase led to an impairment of **Wnt-dependent** acute and chronic myeloid leukemic **stem cells**. Dr Roos believes that 5-lipoxygenase inhibitors might represent a novel type of Wnt inhibitor. **(43)** Based on Dr Jessica Roos’s study, the combination of a COX-2 inhibitor (celebrex) with a 5-LOX inhibitor itraconazole might be synergistic, targeting cancer stem cells through WNT inhibition.(44-46)

5-LOX overexpressed in MCL cells X7
Inhibitors are promising therapeutic strategy for MCL

Dr Robert Boyd reported in 2009 his study, “Protein profiling of plasma membranes defines aberrant signaling pathways in mantle cell lymphoma.” showing that “**5-lipoxygenase (5-LO), a key enzyme in leukotriene biosynthesis, was associated with lipid rafts and was up-regulated ~7-fold in MCL compared with normal B cells**….Inhibitors of 5-LOX activity **induced apoptosis in MCL cell lines** and primary chronic lymphocytic leukemia cells, indicating an important role for the leukotriene biosynthetic pathway in MCL and other B cell malignancies….this could be a **promising therapeutic strategy for MCL and CLL.**“(45)

Countering CD40 Activation of B-Cell Lymphoma with 5-LOX inhibitor

Runarsson et al. observed that treatment of B-CLL cells with a 5-LOX inhibitor **"counteracted CD40-dependent activation of these cells by inhibiting CD40-induced DNA synthesis and CD40-induced expression of CD23, CD54, and CD150"** Runarsson, Gudmundur, et al. "Leukotriene B 4 plays a pivotal role in CD40-dependent activation of chronic B lymphocytic leukemia cells." *Blood* 105.3 (2005): 1274-1279.

Suppressing Lymphangiogenesis

In 2015, Dr Wang studied Malignant Pleural Effusion in mice showing itraconazole inhibits MPE by suppressing lymphangiogenesis, the creation of new lymph vessels by the cancer cells which leads to growth of the tumor mass.(14) Dr Song found that the drug lenalidamide also suppressed lymphangiogeneis in a mouse xenograft model of Mantle cell Lymphoma. (15) Dr Song found that networks of new lymphatics within the tumor mass were inhibited by the drug.(15)

Caution: Concurrent Use with Rituximab (1)

"Concurrent use of ITZ was shown, in vitro and in vivo using a murine xenograft model of lymphoma, to abrogate the therapeutic effect of rituximab"(1)

Cancer Stem cells – Hedgehog pathway

In 2010, Dr James Kim screened a panel of human drugs identifying itraconazole as Hh (Hedgehog) inhibitor with suppression of Hh-dependent tumor growth in vivo at serum levels comparable to those in patients undergoing antifungal therapy.(16) The Hh (Hedgehog) pathway is a cancer stem cell pathway.(17-19) "*Hedgehog regulates cancer stem cells fostering tumorigenesis"*. (18)

Hedgehog Pathway in Mantle Cell Lymphoma

Dr Victoria Campbell published in 2015, "Hedgehog signaling in cancer stem cells: a focus on hematological cancers." Dr CAmpbell says:

> *"Expression of BCL-2 is increased in the presence of active Hh signaling and down-regulated upon inhibition of the pathway….Components of the Hh pathway and key downstream targets (BCL-2 and BCL-XL) are expressed in a variety of NHL (non-Hodgkins Lymphoma) cell lines …Burkitt's Lymphoma cells underwent apoptosis in the absence of Hh signaling both in vitro and in vivo".(20)*

GLi proteins are the downstream effectors of Hh signaling, and were studied by Dr Campbell and found to be upregulated in Mantle Cell Lymphoma. Inhibiting GLi resulted in down-regulation of BCL-2 and Cyclin D1, decreased proliferation and greater sensitivity to drug treatment.(20)

> *"In **mantle cell lymphoma**, a murine model showed **up- regulation of the GLI transcription factors at the gene level**,97 confirming previous work showing the **GLI transcription factors to be over-expressed in mantle cell lymphoma**, both in cell lines and primary lymphoma cells, compared to normal B cells.98 Further, targeting the GLI transcription factors with antisense oligonucleotides **down-regulated BCL-2 and Cyclin D1** resulting in decreased proliferation and increased susceptibility to chemotherapy.(20)*

In 2008, Dr Hegde suggested that molecular targeting the **hedgehog-GLI signaling pathway in mantle cell lymphoma is "** a potential strategy to improve therapy for mantle cell lymphoma." (21)

Itraconazole Inhibits Hh Pathway in Gastric Cancer

22) Dr Hu reported in 2017 that "Itraconazole induces apoptosis and cell cycle arrest **via inhibiting Hedgehog signaling** in gastric cancer cells (in-vitro and in-vivo xenograpt model). Dr Hu reports, *"Itraconazole could remarkably inhibit the proliferation of gastric cancer cells…. In vivo studies demonstrated that itraconazole by oral administration could inhibit the growth of cancer cell xenografts.(in-vivo)"*(22)

Hh Pathway in Breast Cancer

Dr X Wang reported in Cancer Letters 2017, that itraconazole has an anti-proliferative effect on breast cancer by inhibiting the Hedgehog pathway, and inducing apoptosis and autophagic cell death. Dr Wang writes:

> *In breast cancer cell lines, itraconazole induced apoptosis by altering mitochondria membrane potential, reducing BCL-2 expression and elevating caspase-3 activity. Itraconazole also induced autophagic cell death via LC3-II expression upregulation, …Itraconazole treatment inhibited hedgehog pathway key molecular expression, such as SHH and Gli1, resulting in promotion of apoptosis and autophagy. The anti-proliferation effect of itraconazole-induced apoptosis and autophagy via hedgehog pathway inhibition was confirmed… A human xenograft nude mouse model corroborated the anti-breast cancer activity as evidenced by reduced tumor size, and increased tumor tissue apoptosis and autophagy." (23)*

Dr Oladapo reported in 2017 Cancer Letter, *" Activation of the Hedgehog (Hh) pathway effector GLI1 is linked to tumorigenesis and invasiveness in a number of cancers, and pharmacological targeting of GLi1 inhibits proliferation, tumor emboli formation and in vivo tumor growth of inflammatory breast cancer cells."*(24)

Dr Tsubamoto reported on 13 patients with Triple-Negative Breast Cancer with relapsed/refractory metastatic disease treated with combined itraconazole and chemotherapy. Median Overall Survival was 20 months longer than other similar studies using chemotherapy alone.(25)

Dr Richard Kast : Inhibiting hedgehog with itraconazole

Dr Richard Kast in 2017 Oncotarget, writes of itraconazole:(26)

> *" The primary mode of anti-cancer action is inhibition of Hh (Hedgehog) signaling…Itraconazole inhibits release of Gli1 thus keeping it sequestered in the cytoplasm …In preclinical studies itraconazole inhibition of Hh signaling inhibited growth of breast cancer, melanoma, and endometrial cancer".(26)*

Inhibiting AKT/mTOR in Endometrial Cancer

Dr Tsubamoto wrote in 2017 Anticancer Research that Itraconazole Inhibits AKT/mTOR Signaling and Proliferation in Endometrial Cancer Cells. (27) Using an in-vitro model, Dr Tsubamoto found that Itraconazole suppresses the growth of EC (endometrial cancer) cells by inhibiting AKT/mTOR signalling." He wrote:

> *"Itraconazole did not suppress GLI1 or GLI2 transcription but did inhibit the expression of mammalian target of rapamycin (mTOR) signaling components in AN3-CA and HEC-1A cells, while inducing that of microtubule-*

associated protein 1A/1B-light chain 3-II, a marker of autophagy. ATP-binding cassette transporter A1 gene was down-regulated in Ishikawa, HEC-50B and SNG-II cells."

Mantle Cell Lymphoma : mTor Inhibition Synergizes with Ibrutinib,

Dr Li and Wang in In J Cancer 2018 reported ,"*the mTOR kinase inhibitor everolimus synergistically enhances the anti-tumor effect of the Bruton's tyrosine kinase (BTK) inhibitor PLS-123 on Mantle cell lymphoma*".

Targeting the B-cell receptor (BCR) signaling pathway with the Bruton's tyrosine kinase (BTK) inhibitor, **ibrutinib**, has demonstrated favorable therapeutic effects in relapsed/refractory Mantle Cell Lymphoma patients. Dr Li used a "novel irreversible BTK inhibitor, PLS-123, more potent and selective than ibrutinib"**.** Using in vitro screening, Dr Li discovered that the combination of BTK inhibitor drug and an mTOR inhibitor drug (in this case, everolimus) were synergistic in reducing proliferation and motility of Mantle Cell Lymphoma (in vitro). He says:

"Simultaneous inhibition resulted in marked induction of apoptosis and cell cycle arrest in the G1 phase, which were accompanied by upregulation of pro-apoptotic proteins (cleaved Caspase-3, cleaved PARP and Bax), repression of anti-apoptotic proteins (Mcl-1, Bcl-xl and XIAP), and downregulation of regulators of the G1/S phase transition (CDK2, CDK4, CDK6 and Cyclin D1). Gene expression profile analysis revealed simultaneous treatment with these agents led to inhibition of the JAK2/STAT3, AKT/mTOR signaling pathways and SGK1 expression. Finally, the anti-tumor and pro-apoptotic activities of combination strategy have also been demonstrated using xenograft mice models. ***Taken together, simultaneous suppression of BTK and mTOR may be indicated as a potential therapeutic modality for the treatment of MCL.****"(29)*

These findings suggest probably synergy of itraconazole, another potent mTor inhibitor, with ibrutinib.

Addition of mTOR inhibitor : Reverses drug resistance and enhances ibrutinib activity

Dr Xiaohong Zhao studied acquired Ibrutinib resistance in Mantle Cell Lymphoma, reporting in2017.(30) Dr Xiaohong Zhao says that ibrutinib has high response rates in B-cell lymphomas. However, ibrutinib resistance and disease relapse with fulminant progression is a growing problem. Dr Xiaohong Zhao studied the tumour microenvironment (TME) influence on ibrutinib efficay, and acquired ibrutinib resistance, and found that:

"MCL cells develop ibrutinib resistance through evolutionary processes driven by ***dynamic feedback between MCL cells and TME(tumor micro environment)****, leading to kinome adaptive reprogramming, bypassing the effect of ibrutinib and reciprocal activation of* ***PI3K-AKT-mTOR*** *and integrin-β1 signalling.* ***Combinatorial disruption of B-cell receptor signalling*** *(ibrutnib)* ***and PI3K-AKT-mTOR axis*** *(itraconazole)* ***leads to release of MCL cells from TME, reversal of drug resistance and enhanced anti-MCL activity in MCL patient samples and patient-derived xenograft models****."(30)*

Note: Itraconazole profoundly inhibits mTOR, so combination with ibrutinib would potentially overcome TME acquired resistance.

Pterostilbene also inhibits mTOR in Mantle Cell Lymphoma

See: Yu, Dandan, et al. "Targeting the PI3K/Akt/mTOR signaling pathway by pterostilbene attenuates mantle cell lymphoma progression." Acta biochimica et biophysica Sinica 50.8 (2018): 782-792.

Examining the Tumor Micro-Environment Niche in Mantle cell Lymphoma – The "CD40L + Ck" coculture model – Targeting Bcl-xL with BTK inhibitor and Type II anti-CD20

Dr Chiron studied the micro-environemnt in Mantle Cell Lymphoma and its effect on resistance to drug treatment with ibrutinib.(33-35) Dr. Choron found that "**despite a significant level of the proliferation index Ki67 in LN (lymph node mantle cells) , we did not detect any proliferating peripheral blood (PB) MCL cells,** suggesting a major role of the tumor ecosystem." In other words, once the mantle cell lymphoma cells left the lymph node and appeared in circulation, they were no longer proliferating. The tumor micro-environment in the lymph node was responsible for molecular signalling with CD40L and Cytokines which upregulated genetic signatures for proliferation and migration in the Mantle Cells.

Dr Chiron's lab cultured 21 MCL (Mantle Cell) samples and found that CD40L induced cell-cycle progression, which was amplified by a MCL-specific cytokine cocktail such as IL-6, IL-10 etc.(Ck).(33) Dr Chiron's lab then performed RNA-sequencing in MCL cells from (peripheral blood) (PB) or cocultured and compared gene expression . They found more than 65% of genes induced in the "CD40L + Ck" coculture model are also upregulated in the LN (lymph node) compared to PB (peripheral blood). The co-culture model revealed upregulation of molecular signatures characteristic of MCL such as cell cycle, BCR, NFkB/NIK and the anti-apoptotic Bcl2 family.

> *"We first observed that microenvironment signalings resulted in an unbalanced regulation of anti- and pro-apoptotic proteins in MCL, but not in normal B cells (CD19 + CD5+). The major regulation was an increase in expression of Bcl-xL associated with a downregulation of Bim and Noxa."*
>
> "Using the functional BH3-profiling assay, we demonstrated that, whereas PB MCL cells are dependent on Bcl2 for survival, Bcl-xL upregulation was responsible for loss of mitochondrial priming and resistance. Consequently, **whereas Bcl2 BH3-mimetic efficiently triggered apoptosis in PB MCL, cells protected by the microenvironment were resistant."**
>
> *"We then hypothesized that targeting Bcl-xL could increase treatment efficacy. Using our coculture model, we developed efficient targeted strategies (i.e., BTK inhibitor, Type II anti-CD20), which counteract Bcl-xL overexpression and overcome drug resistance in primary cells ex vivo. This strategy should target cells protected into their niches and our ongoing Trial (NTC#02558816) will rapidly determine in vivo efficacy in MCL."*

Review 2017

Dr R Pounds wrote his paper in 2017 Oncology Letter entitled," Repurposing itraconazole for the treatment of cancer. (28) He says:

> *"Itraconazole is a broad-spectrum anti-fungal agent which acts via several mechanisms to prevent tumour growth, including inhibition of the Hedgehog pathway, prevention of angiogenesis, decreased endothelial cell proliferation, cell cycle arrest and induction of auto-phagocytosis…. These allow itraconazole… to increase drug efficacy and overcome drug resistance."(28)*

Itraconazole Inhibits HH, WNT Akt in Cerv Cancer Cells

Dr Tomoko Ueda reported in Anticancer Research 2017, the effect of itraconazole on cervical cancer cells finding it "modulates Hedgehog, WNT/β-catenin, as well as Akt Signalling, and Inhibits Proliferation" (31) He says

itraconazole treatment caused:

> *"**8-fold down-regulation** in the expression of GLI1 (Hedgehog), WNT4 and WNT10A among itraconazole-treated CaSki (Cervical Cancer) cells. Immunoblots showed suppression in β-catenin expression and Akt phosphorylation."(31)*

Itraconazole Inhibits VEGF

Benjamin Nacev reported in 2011 that itraconazole has potent anti-angiogenic activity by inhibiting "*vascular endothelial growth factor receptor 2 (VEGFR2) glycosylation, trafficking, and signaling in endothelial cells*."(32) itraconazole significantly inhibited the binding of vascular endothelial growth factor (VEGF) to VEGF receptor 2 (VEGFR2) .(32)

Phase 2 trial for Basal Skin Cancer Itraconazole (66)

Above image: Patient with large basal cell cancer on scalp before (red arrow) and after 59 days of itraconazole 400 mg daily(green arrows) . Courtesy of Daniel Kim, 2014 (66) C= vertex Views. D= side Views.

A Phase Two Trial of oral itraconazole for Basal Cell carcinoma by Dr Daniel Kim in 2014 yielded impressive results in 29 patients (66) (see above image) Skin Cancers, both basal cell and squamous cell show upregulation of the Hedgehog pathway which is inhibited by itraconazole. (54-56)

FDA Boxed Warning for Itraconazole in patients with CHF :

> *Congestive Heart Failure, Cardiac Effects and Drug Interactions: Itraconazole capsules should not be administered for the treatment of* ***onychomycosis*** *in patients with evidence of* ***ventricular dysfunction such as congestive heart failure (CHF) or a history of CHF.***

Mebendazole Antiparasitic Drug repurposed as Anti-cancer Drug

Another Hedgehog inhibitor is mebendazole as discussed in my previous article.(57-65) Usual dosage is 100 mg caps one cap twice a day.

Where to purchase *mebendazole:*
Ask for Brad at: Pavilion Compounding pharmacy
3193 Howell Mill Road NW , Suite 122A
Atlanta, GA 30327
(404) 350-5780

Conclusion: The repurposed anti-fungal drug Itraconazole, is also an impressive anti-cancer drug. Hopefully, itraconazole will be incorporated into mainstream oncology on a routine basis in the near future.

Articles with Related Interest:

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Links and References

1) Pantziarka, Pan, et al. “Repurposing Drugs in Oncology (ReDO)—itraconazole as an anti-cancer agent.” ecancermedicalscience 9 (2015). free pdf

ITZ is commonly available as a generic, prescription-only drug. Common trade names include Sporanox (Janssen) and Onmel (Merz).
ITZ is most commonly administered orally, either as 100 mg or 200 mg capsules or as oral solution. It can also be administered intravenously, though this route is less commonly used. Dosing varies by indication; generally it is used in the range **100 mg–600 mg daily**, for between one to 30 days. It is also used for long-term maintenance or prophylaxis, for example **200 mg**–400 mg daily for HIV-infected patients, 400 mg daily for patients suffering from chronic pulmonary aspergillosis, or 100 mg per day for more than one year for the treatment of paracoccidioidomycosis [1].

for example 400 mg/day for one year for the treatment of severe blastomycosis in children)
Analysis showed that the levels required for an **anti-angiogenic response could also be achieved using a 200 mg oral dose of the drug.**

The authors estimated that the human doses of ITZ required to achieve the serum levels achieved in these murine models is in the range of 600–900 mg/day, a high dose but one which has been used clinically for long periods in humans

Evidence has also been produced that indicates that clinicians may need to exercise caution in the use of ITZ therapy in patients being treated with monoclonal antibodies. The monoclonal antibody **rituximab** is an effective therapy for diseases characterised by CD20-expressing B cell dysfunction or over-expression, including lymphomas, leukaemias, and auto-immune conditions. Concurrent use of ITZ was shown, in vitro and in vivo using a murine xenograft model of lymphoma, to abrogate the therapeutic effect of rituximab [25]. In vitro analysis suggested that this effect of ITZ was specific to lipid-raft-associated molecules, and ITZ impaired alemtuzumab-induced cell death in a dose dependent manner.

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Itraconazole

Double Hit Lymphoma !!!!!!!!!!!!!!!!!!!!

combined **itraconazole** with ABT199 (**Venetoclax**) or JQ1 (**c-Myc inhibitor)** had a strong synergistic efficacy. **Ibrutinib also.**

2) Itraconazole, an Oral Antifungal Drug, Inhibits Tumor Growth and Enhances Therapeutic Agent Activity in Double Hit Lymphoma
Juan J Gu, Lianjuan Yang, Benjamin Jarmusz, Daniel Cress, Cory Mavis, Matthew J. Barth and Francisco J. Hernandez-Ilizaliturri
Blood 2016 128:5380;

Background: Among B-cell lymphoma, double hit lymphoma (DHL) is defined by a MYC/8q24 combined with another recurrent BCL/18q21 breakpoint. DHL most affects elderly patients (median age ranged from 51 to 65 years) and have a poor prognosis with a median overall survival of 0.2-1.5 year. R+CHOP or high-intensity treatment including high-dose chemotherapy followed stem cell transplant yield to suboptimal outcomes. Therefore, there is a need to discover alternative effective agents. Itraconazole, an oral antifungal drug, was

reported had potent anticancer activity in **basal cell carcinoma, non-small cell lung cancer and prostate cancer** by interfering with the apoptotic threshold of cancer cells. Its activity in DHL has yet to be defined.

Methods: Double hit lymphoma cell lines: DOHH2, VAL and ROS50 were exposed to **escalating doses of itraconazole (0-20μM) for 24, 48 and 72h.** Changes in cell viability and cell cycle distribution were evaluated using the Presto Blue assay and flow cytometry respectively. IC50 was calculated by Graphpad Prism 6 software. Loss of ATP, Caspase 3/7 activation and loss of mitochondrial membrane potential (??m) following itraconazole exposure were assessed by Cell Titer Glo, Caspase3 /7 detection kit and DiOC6 staining, respectively. DHL cells were exposed to itraconazole or vehicle combined with various inhibitors, such as **BCL-2 inhibitor ABT199, c-MYC inhibitor JQ1, bruton's kinase inhibitor ibrutinib** and different generations of **proteasome inhibitors** (carfilzomib or MLN4924) for 48h and cell viability was determined by Presto Blue assay. Coefficient of synergy index was calculated using the CalcuSyn software.

Result: Itraconazole consistently showed **potent, specific, dose-and time- dependent inhibition in DHL**. In vitro exposure cells to itraconazole **resulted in a loss of ATP, loss of mitochondrial membrane potential and activation of caspase 3/7 activities. Itraconazole caused G1 cell cycle arrest and decrease S phase in DHL**. Moreover, itraconazole had a **strong synergistic anti-tumor effect** combined with various inhibitors, including **BCL-2 inhibitor ABT199, c-MYC inhibitor JQ1**, **bruton's kinase inhibitor ibrutinib** and different generations of proteasome inhibitors (**bortezomib**, carfilzomib, Ixazomib or MLN4924).

Conclusion: Taking together, our data suggest that itraconazole had a potent anti-tumor activity against DHL. Disruption of mitochondrial membrane potential and activation of caspases may contribute its antitumor activity against DHL. It is worth noticing that combined itraconazole with DHL specific target inhibitors ABT199 (Venetoclax) or JQ1 had a strong synergistic efficacy. Our findings offered the first assessment of the efficacy of itraconazole as a novel anticancer agent in double hit lymphoma. The molecular mechanism of action of itraconazole need to be further investigated in the near further.

B Cell NHL !!!!!!!!!!!!!!!!!!!!!!!!!!!!!!

3) Gu, Juan J., et al. "Itraconazole, an Oral Antifungal Drug, Is Active in Chemotherapy Resistant B-Cell Non-Hodgkin Lymphoma and Enhances the Anti-Tumor Activity of Chemotherapy Agents." Blood. (2016): 5138-5138.

Background: Relapsed/refractory diffuse large B-cell lymphoma (DLBCL) patients previously treated with rituximab-based therapy have poor clinical outcome, according to the results from collaborative trial in relapsed aggressive lymphoma (CORAL) study. It stresses the need to identify and/or optimize novel targeted agents. To better understand the molecular mechanisms underlining the acquired resistance to rituximab, we generated and characterized several **rituximab-resistant DLBCL cell lines (RRCLs)**. Itraconazole, an oral antifungal agent, was reported had novel anticancer activity in basal cell carcinoma, non-small cell lung cancer and prostate cancer. In our current work, we define and characterize the anticancer activity of itraconazole in preclinical rituximab-sensitive or -resistant lymphoma models.

Methods: A panel of **rituximab-sensitive (RSCL) and rituximab-resistant (RRCL) cell lines** were exposed to escalating doses of **itraconazole (0-20μM) for 24, 48 and 72h**. Changes in cell viability and cell cycle distribution were evaluated using the Presto Blue assay and flow cytometry respectively. IC50 was calculated by Graphpad Prism6 software. Loss of mitochondrial membrane potential (??m) following itraconazole exposure was assessed by DiOC6 and flow cytometry. Subsequently lymphoma cells were exposed to itraconazole or vehicle and various chemotherapy agents such as doxorubicin (1μM), dexamethasone (1μM), cDDP (20μg/ml), bortezomib (20nM), carfilzomib (20nM) or MLN2238 (20nM) for 48 hours. Coefficient of synergy was calculated using the CalcuSyn

software. Changes in hexokinase II (HKII), Voltage dependent anion channel protein (VDAC), LC3 and BCL-xL expression levels were determined by western blotting after exposure cells to itraconazole. VDAC-HKII interactions following in vitro exposure to itraconazole were determined by immunoprecipitation of VDAC and probing for HKII in RSCL and RRCLs.

Result:**Itraconazole consistently showed potent, specific, dose-and time- dependent inhibition of all our sensitive and resistant lymphoma cell lines.** In vitro exposure cells to itraconazole resulted in a **loss of mitochondrial membrane potential and caused G2 cell cycle arrest. (arrest of the cell in G_2 just before mitotic entry)**

Itraconazole significantly had a synergistic anti-tumor effect combined with various chemotherapeutic agents, including doxorubicin, dexamethasone, cisplatin and different generations of proteasome inhibitors (**bortezomib**, carfilzomib or ixazomib) in both RSCL and RRCL. Western blot and immunoprecipitation studies demonstrated that following exposure to itraconazole, **HKII bound less to mitochondrial specific protein VDAC.** Complete silencing of HKII (using HKII siRNA interference) resulted in a rescue of loss in the mitochondrial membrane potential induced by intraconazole.

Conclusion: Taking together, our data suggest that itraconazole had a potent anti-tumor activity against rituximab-sensitive or resistant pre-clinical models. The disruption of HKII from mitochondria following itraconazole exposure may contribute to lower the mitochondrial membrane potential and enhance the chemotherapeutic efficacy. Our finding highlights itraconazole as a **potential therapeutic agent in the treatment of B-cell malignancies,** and strongly supports clinical translation of its use.

2017 Nice review !!!!!!!!!!!!!!!!!!! Nice chart images !!!!

4) Tsubamoto, Hiroshi, et al. “Repurposing itraconazole as an anticancer agent.” Oncology Letters 14.2 (2017): 1240-1246.

Itraconazole, a common anti-fungal agent, has demonstrated potential anticancer activity, including:

1. reversing chemoresistance mediated by P-glycoprotein,
2. modulating the signal transduction pathways of Hedgehog,
3. mechanistic target of rapamycin (mTOR)
4. and Wnt/β-catenin in cancer cells,
5. inhibiting angiogenesis and lymphangiogenesis, and
6. possibly interfering with cancer-stromal cell interactions.

Clinical trials have suggested the clinical benefits of itraconazole monotherapy for **prostate cancer and basal cell carcinoma,** as well as the survival advantage of combination chemotherapy for **relapsed non-small cell lung, ovarian, triple negative breast, pancreatic and biliary tract cancer**.

anti-angiogenic agent in 2007
inhibitor of Hedgehog signaling in 2010
Itraconazole inhibits AKT (protein kinase B)/mechanistic target of rapamycin (mTOR) signaling
in human umbilical vein endothelial cells (HUVECs),
glioblastoma,
endometrial carcinoma (EC) and
melanoma cells

Inhibition of Hedgehog signaling was observed in basal cell carcinoma, medulloblastoma, pleural mesothelioma, breast cancer and melanoma cells (9,14–17), but not in EC cells (13). **Inhibition of Wnt/β-catenin** signaling was observed in basal cell and examined in melanoma cells (14). **Itraconazole also induced autophagic cell death** in medulloblastoma cells as well as in breast cancer cells (12,17), and **suppressed lymphangiogenesis** in lung carcinoma cells (18).

In HUVECs, itraconazole induced the accumulation of immature N-glycans on VEGFR2, which in turn inhibited autophosphorylation and downstream activation (19); itraconazole also exhibited synergistic effects with bevacizumab, a humanized monoclonal antibody against VEGF (20). Additionally, hypoglycosylation of the epidermal growth factor receptor was observed in renal cell carcinoma cells (19).

Itraconazole directly binds to the mitochondrial protein voltage-dependent anion channel 1 (VDAC1) and interferes with mitochondrial ATP production, leading to the **activation of the AMP-activated protein kinase pathway and the subsequent inhibition of mTOR activity** (11).

Cancer-associated fibroblasts (CAFs) are essential for tumor growth (29). **Itraconazole inhibited the proliferation of CAFs established from human colon cancer cells**, as well as the **secretion of monocyte chemoattractant protein-1** (20). Monocyte/macrophage marker CD14 is a glycosylphosphatidylinositol-anchored glycoprotein present in cholesterol-rich lipid rafts, which contain a variety of signaling proteins and receptors (30,31). In mouse macrophages, itraconazole treatment altered the N-glycosylation of CD14, and increased CD14 transcription and protein expression (32).

Clinical Trials show survival advantage

Itraconazole may be a promising agent for targeting **CSCs** in relapsed disease of multiple types of cancer;

1) itraconazole reverses chemoresistance in cancer cells overexpressing P-gp

2) Itraconazole inhibited the proliferation of CAFs (cancer associated fibroblasts)

3) Itraconazole directly binds to the mitochondrial protein voltage-dependent anion channel 1 (VDAC1) and interferes with mitochondrial ATP production, leading to the activation of the AMP-activated protein kinase pathway and the subsequent inhibition of mTOR activity

4) itraconazole inhibited intracellular cholesterol trafficking to the plasma membrane by binding to Niemann-Pick C1 protein, resulting in cholesterol depletion

5) Itraconazole may be a promising agent for targeting CSCs in relapsed disease of multiple types of cancer

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VDAC

5) Head, Sarah A., et al. "Antifungal drug itraconazole targets VDAC1 to modulate the AMPK/mTOR signaling axis in endothelial cells." *Proceedings of the National Academy of Sciences* 112.52 (2015): E7276-E7285. Antifungal drug itraconazole targets VDAC1 to modulate the AMPK/mTOR signaling axis in endothelial cells.

Itraconazole, a clinically used antifungal drug, was found to possess **potent antiangiogenic and anticancer activity** that is unique among the azole antifungals. Previous mechanistic studies have shown that itraconazole inhibits the **mechanistic target of rapamycin (mTOR) signaling pathway,** which is known to be a **critical regulator of endothelial cell function and angiogenesis.** However, the molecular target of itraconazole that mediates this activity has remained unknown. Here we identify the major target of itraconazole in endothelial cells as the mitochondrial protein voltage-dependent anion channel 1 (**VDAC1**), which regulates mitochondrial metabolism by controlling the passage of ions and small metabolites through the outer mitochondrial membrane. **VDAC1 knockdown profoundly inhibits mTOR activity and cell proliferation in human umbilical vein cells (HUVEC),** uncovering a previously unknown connection between VDAC1 and mTOR. Inhibition of VDAC1 by itraconazole disrupts mitochondrial metabolism, leading to an increase in the cellular AMP:ATP ratio and **activation of the AMP-activated protein kinase (AMPK), an upstream regulator of mTOR.** VDAC1-knockout cells are resistant to AMPK activation and mTOR inhibition by itraconazole, demonstrating that VDAC1 is the mediator of this activity. In addition, another known VDAC-targeting compound, erastin, also activates AMPK and inhibits mTOR and proliferation in HUVEC. **VDAC1 thus represents a novel upstream regulator of mTOR signaling in endothelial cells and a promising target for the development of angiogenesis inhibitors.**

we synthesized a photoaffinity probe of itraconazole to identify its binding proteins from live endothelial cells. we identified voltage-dependent anion channel 1 (VDAC1) as a primary binding protein of itraconazole.

We demonstrated that itraconazole not only **binds directly to VDAC1 but also interferes with its primary cellular function of regulating mitochondrial metabolism**, causing a **drop in cellular energy levels that triggers the energy-sensing protein AMP-activated protein kinase (AMPK)**. Subsequently, **AMPK down-regulates mTOR activity** through direct phosphorylation of the regulatory-associated protein of mTOR (raptor), ultimately leading to **inhibition of endothelial cell proliferation.**

Inhibition of mTOR signalling-Simultanous VDAC and Cholesterol traficing inhibition

Mitochondria are critical for ATP production, and many small molecules that activate AMPK, including metformin, resveratrol, berberine, and rotenone, have been shown to inhibit mitochondrial function (50–54).

The widely prescribed, AMPK-activating antidiabetic drug **metformin has been shown to inhibit angiogenesis in vitro and in vivo** (55) and currently is being evaluated in several clinical trials for various types of cancer (56). However, the **concentrations of metformin required** to activate AMPK in HUVEC **are at least 1,000 times higher** than those required of itraconazole (in the range of low millimoles) (55), suggesting that **itraconazole might be significantly more effective than metformin at inhibiting angiogenesis in patients**. Another drug in trials for cancer, the natural product **curcumin**, also has been shown to activate AMPK and inhibit mTOR (57–59). Interestingly, a recent study demonstrated that, similar to itraconazole, curcumin also interferes with VDAC1 function (60).

6) Head, Sarah A., et al. "Simultaneous Targeting of NPC1 and VDAC1 by Itraconazole Leads to Synergistic Inhibition of mTOR Signaling and Angiogenesis." *ACS chemical biology* 12.1 (2016): 174-182. Simultaneous targeting of NPC1 and VDAC1 by itraconazole leads to synergistic inhibition of MTOR signaling and angiogenesis

The antifungal drug itraconazole was recently found to exhibit **potent antiangiogenic activity** and has since been repurposed as an investigational anticancer agent. Itraconazole has been shown to exert its antiangiogenic activity through **inhibition of the mTOR signaling pathway**, but the molecular mechanism of action was unknown. We recently identified the mitochondrial protein VDAC1 as a target of itraconazole and a mediator of its activation of AMPK, an upstream regulator of mTOR. However, VDAC1 could not account for the previously reported inhibition

of cholesterol trafficking by itraconazole, which was also demonstrated to lead to mTOR inhibition. In this study, we demonstrate that **cholesterol trafficking inhibition by itraconazole is due to direct inhibition of the lysosomal protein NPC1.** We further map the binding site of itraconazole to the sterol-sensing domain of NPC1 using mutagenesis, competition with U18666A, and molecular docking. Finally, we demonstrate that **simultaneous AMPK activation and cholesterol trafficking inhibition leads to synergistic inhibition of mTOR, endothelial cell proliferation, and angiogenesis.**

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7) Anticancer Res. 2017 Jul;37(7):3521-3526. Itraconazole Modulates Hedgehog, WNT/ß-catenin, as well as Akt Signalling, and Inhibits Proliferation of Cervical Cancer Cells. Ueda T1, Tsubamoto H2,3, Inoue K1, Sakata K1, Shibahara H1, Sonoda T3.

Repurposing itraconazole as an anticancer agent has been evaluated in several studies. The present study investigated whether itraconazole exerts an anticancer effect on **cervical cancer cells.**
MATERIALS AND METHODS: **CaSki and HeLa cells** were cultured in itraconazole and vehicle after which colony-forming and cell viability assays were performed. Transcription and protein expression were assessed by cDNA microarray analysis and immunoblotting, respectively.
RESULTS:Itraconazole suppressed proliferation of CaSki and HeLa cells in a dose- and time-dependent manner. Furthermore, CaSki cells were more significantly affected by itraconazole than HeLa cells. The microarray analysis showed an **8-fold down-regulation in the expression of GLI1, WNT4 and WNT10A among itraconazole-treated CaSki cells.** Moreover, the transcription of sterol carrier protein-2 and ATP-binding cassette transporter-1 was unaffected by itraconazole. I**mmunoblots showed suppression in ß-catenin expression and Akt phosphorylation.**
CONCLUSION:Itraconazole is a multi-targeting anticancer agent and a promising therapeutic agent for cervical cancer.

Prostate Cancer

8) Antonarakis, Emmanuel S., et al. “Repurposing itraconazole as a treatment for advanced prostate cancer: a noncomparative randomized phase II trial in men with metastatic castration-resistant prostate cancer.” *The oncologist* 18.2 (2013): 163-173.Repurposing Itraconazole as a Treatment for Advanced Prostate Cancer: A Noncomparative Randomized Phase II Trial in Men With Metastatic Castration-Resistant Prostate Cancer

Background. The antifungal drug itraconazole inhibits angiogenesis and Hedgehog signaling and **delays tumor growth in murine prostate cancer xenograft models.** We conducted a noncomparative, randomized, phase II study evaluating the antitumor efficacy of two doses of oral itraconazole in men with metastatic prostate cancer.

Patients and Methods. We randomly assigned 46 men with chemotherapy-naïve metastatic castration-resistant prostate cancer (CRPC) to receive **low-dose (200 mg/day) or high-dose (600 mg/day) itraconazole** until disease progression or unacceptable toxicity. The primary endpoint was the prostate-specific antigen (PSA) progression-free survival (PPFS) rate at 24 weeks; a 45% success rate in either arm was prespecified as constituting clinical significance. Secondary endpoints included the progression-free survival (PFS) rate and PSA response rate (Prostate Cancer Working Group criteria). Exploratory outcomes included circulating tumor cell (CTC) enumeration, serum androgen measurements, as well as pharmacokinetic and pharmacodynamic analyses.

Results. The high-dose arm enrolled to completion (n = 29), but the low-dose arm closed early (n = 17) because of a prespecified futility rule. The **PPFS rates at 24 weeks were 11.8% in the low-dose arm and 48.0% in the**

high-dose arm. The median PFS times were 11.9 weeks and 35.9 weeks, respectively. PSA response rates were 0% and 14.3%, respectively. In addition, itraconazole had favorable effects on CTC counts, and it suppressed Hedgehog signaling in skin biopsy samples. Itraconazole did not reduce serum testosterone or dehydroepiandrostenedione sulfate levels. Common toxicities included fatigue, nausea, anorexia, rash, and a syndrome of hypokalemia, hypertension, and edema.

Conclusion. **High-dose itraconazole (600 mg/day) has modest antitumor activity in men with metastatic CRPC** that is not mediated by testosterone suppression.
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immunosuppressive effects

9) Int J Immunopharmacol. 1991;13(2-3):299-304.
Comparison of the immunosuppressive activities of the antimycotic agents itraconazole, fluconazole, ketoconazole and miconazole on human T-cells. Pawelec G1, Ehninger G, Rehbein A, Schaudt K, Jaschonek K.

Four antifungal agents have been screened in vitro for their immunosuppressive effects on proliferative responses in human mixed lymphocyte cultures (MLC). A hierarchy of inhibitory activity was observed, where itraconazole was greater than ketoconazole greater than miconazole greater than fluconazole, with itraconazole as suppressive as cyclosporin A, and fluconazole completely without suppressive activity. The mechanism of inhibition did not involve blockade of T-cell growth factor production and, consistent with this, **interleukin-2-dependent T-cell clone proliferation was blocked by these agents** in the same order of decreasing activity as in MLC. The secretion of cytokines without known T-cell growth factor activity (interferon-gamma, tumour necrosis factor-alpha) was also not significantly blocked by these agents. These results therefore demonstrate that antifungal azole drugs may be variably strongly immunosuppressive for human T-lymphocyte proliferation in vitro, but none appear to be so via a mechanism involving inhibition of cytokine secretion.

10) J Toxicol Sci. 1994 Feb;19(1):7-15.
The effects of itraconazole on the immune responses in ICR mice.
Kim JH1, Ahn YK.
Effects of itraconazole (ICZ) on the immune responses were studied in ICR mice. Mice were divided into 5 groups (10 mice/group), and ICZ at doses of 10, 20, 40 and 80 mg/kg were orally administered to mice once a day for 21 days. Mice were immunized and challenged with sheep red blood cells (SRBC). The body weight gains and the relative weights of spleen and thymus were dose-dependently increased following ICZ treatment. However, Plaque forming cells (PFC) and hemagglutination (HA) titers to SRBC were significantly suppressed in mice doses at 80 mg/kg ICZ, as compared with those in controls. Delayed-type hypersensitivity (DTH) reaction to SRBC, phagocyte activity and circulating leukocytes also were significantly decreased in mice dosed at 40 and 80 mg/kg ICZ. These studies demonstrate that **ICZ treatment results in a marked suppression in both humoral and cell-mediated immune responses to SRBC** at concentrations producing embryotoxicity.

11) Int J Immunopharmacol. 1992 Aug;14(6):1011-7.
Lack of immunosuppression by ketoconazole and itraconazole.
Cools M1, Aerts F, Van Wauwe J.

The antifungal drugs ketoconazole and itraconazole were evaluated for their effects in the following test systems: in vitro, phytohaemagglutinin (PHA)-induced proliferation of human peripheral blood mononuclear cells and **IL-2-driven proliferation of CTLL-2 cells**; in vivo, antibody response to sheep red blood cells (SRBC) and delayed-type hypersensitivity (DTH) reaction to oxazolone. At a concentration of 10 microM, ketoconazole moderately and

itraconazole strongly inhibited thymidine (Thd) incorporation in human peripheral blood mononuclear cells cultured in medium supplemented with 5% human serum. Increasing the serum concentration from 5 to 20% almost completely reversed these inhibitory effects. Also, cell viability, found to be less than 15% in cultures containing 10 microM itraconazole was restored by increasing the serum concentrations in the culture medium. Similar observations were made in experiments using IL-2-stimulated CTLL-2 cells: the growth inhibition in the presence of 10 microM ketoconazole or 1 microM itraconazole could be counteracted by increased serum supplementation. In vivo, subchronic intraperitoneal dosing with 40 mg/kg ketoconazole or itraconazole to mice had no effect on the antibody response to SRBC as measured by the number of splenic IgM and IgG plaque-forming cells and did not significantly affect the DTH response to oxazolone. **These data indicate that neither ketoconazole nor itraconazole exert immunosuppressive properties in vivo. Their in vitro inhibitory effects on PHA-induced lymphocyte proliferation and IL-2-dependent CTLL-2 growth are reversed by the serum supplementation to the culture medium and these activities should therefore be considered as in vitro artefacts.**

12) The effects of amphotericin B, fluconazole and miconazole on neutrophil and lymphocyte function in a guinea pig model
D. C. Drummond C. W. Wong L. M. Whitman J. G. McCormack
Journal of Antimicrobial Chemotherapy, Volume 36, Issue 2, 1 August 1995, Pages 375–384,

We studied the effects of amphotericin B, fluconazole and miconazole on guinea pig neutrophil and lymphocyte function. Neutrophil adherence, chemotaxis, and deoxyglucose uptake and mitogen-induced lymphocyte proliferation were examined. The drugs were administered intraperitoneally in varying dosages based on those used therapeutically, either as a single infusion or daily for 3 days. Miconazole at high dosage (60 mg/kg) suppressed mitogen-induced lymphocyte proliferation, otherwise a single dose of any of the drugs had no effect on neutrophil or lymphocyte function irrespective of concentration used. Variable stimulative or suppressive effects on neutrophil and lymphocyte function were observed after three daily doses of each drug, but there was no dose-response pattern and the effects were erratic. **The data show that, contrary to previous findings in vitro, amphotericin B, fluconazole and miconazole were not consistently immunosuppressive in vivo in this animal model.**

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itraconazole – potent inhibitor of 5-LOX

itraconazole – potent inhibitor of 5-LOX

13) Jaschonek, K., et al. "5-Lipoxygenase inhibition by antifungal azole derivatives: new tools for immunosuppression?." Eicosanoids 2.3 (1989): 189-190.

The effect of novel azole derivatives (itraconazole and fluconazole) on 5-lipoxygenase activity was examined using human polymorphonuclear leukocytes (PMNL) as the enzyme source. The novel imidazole derivative **itraconazole proved to be a potent inhibitor (IC50 2 X 10(-6) M) of 5-lipoxygenase activity.** In contrast, fluconazole, an antifungal agent with bistriazole structure, had no effect on the Ca2+ ionophore A 23187-induced formation of 5-lipoxygenase metabolites in PMNL.

Itraconazole inhibits lymphangiogenesis

14) Wang, Yunfen, et al. "Itraconazole can inhibit malignant pleural effusion by suppressing lymphangiogenesis in mice." Translational lung cancer research 4.1 (2015): 27. our study substantiate the therapeutic effect of

itraconazole on MPE in vivo, largely through inhibiting lymphangiogenesis in the generation and progression of MPE.

Lenalidamide inhibits lymphangiogenesis Mantle Cell

15) Cancer Res. 2013 Dec 15; 73(24): 7254–7264.
Lenalidomide inhibits lymphangiogenesis in preclinical models of mantle cell lymphoma Kai Song,1 Brett H. Herzog,1 Minjia Sheng,1,2 Jianxin Fu,1,3,4 J. Michael McDaniel,1 Jia Ruan,4,5 and Lijun Xia1,3,4,6

Lymphomas originate in and spread primarily along the lymphatic system. However, whether lymphatic vessels contribute to the growth and spreading of lymphomas is largely unclear. Mantle cell lymphoma (MCL) represents an aggressive non-Hodgkin's lymphoma. We found that MCL **exhibited abundant intratumor lymphatic vessels**. Our results demonstrated that the immunomodulatory drug lenalidomide potently **inhibited the growth and dissemination of MCL in a xenograft MCL mouse model,** at least in part, by **inhibiting functional tumor lymphangiogenesis**. Significant numbers of tumor-associated macrophages expressing vascular endothelial growth factor-C were found in both human MCL and mouse MCL xenograft samples. Lenalidomide treatment **resulted in a significant reduction in the number of MCL-associated macrophages**. In addition, in vivo depletion of monocytes/macrophages impaired functional tumor lymphangiogenesis and inhibited MCL growth and dissemination. Taken together, our results indicate that tumor lymphangiogenesis contributes to the progression of MCL and that lenalidomide is effective in decreasing MCL growth and metastasis most likely by **inhibiting recruitment of MCL-associated macrophages.**

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Cancer Stem cells – Hedgehog pathway

16) Kim, James, et al. "Itraconazole, a commonly used antifungal that inhibits Hedgehog pathway activity and cancer growth." Cancer cell 17.4 (2010): 388-399.
Itraconazole identified as Hh pathway inhibitor in screen of human-experienced drugs Itraconazole appears to act on Smoothened at distinct site from cyclopamine Itraconazole inhibits accumulation of Smoothened in primary cilium Itraconazole suppresses Hh-dependent tumor growth in vivo

In a screen of drugs previously tested in humans we identified itraconazole, a systemic antifungal, as a **potent antagonist of the Hedgehog (Hh) signaling pathway** that acts by a mechanism distinct from its inhibitory effect on fungal sterol biosynthesis. Systemically administered itraconazole, like other Hh pathway antagonists, can suppress Hh pathway activity and the growth of medulloblastoma in a mouse allograft model and does so **at serum levels comparable to those in patients undergoing antifungal therapy.** Mechanistically, itraconazole appears to act on the essential Hh pathway component Smoothened (SMO) by a mechanism distinct from that of cyclopamine and other known SMO antagonists, and prevents the ciliary accumulation of SMO normally caused by Hh stimulation.

17) Takebe, Naoko, et al. "Targeting Notch, Hedgehog, and Wnt pathways in cancer stem cells: clinical update." Nature reviews. Clinical oncology 12.8 (2015): 445.

18) Coni, Sonia, Paola Infante, and Alberto Gulino. "Control of stem cells and cancer stem cells by Hedgehog signaling: pharmacologic clues from pathway dissection." Biochemical pharmacology 85.5 (2013): 623-628.

Hedgehog is a key morphogen regulating embryonic development and tissue repair. Remarkably, when misregulated, it leads to tumorigenesis. Hedgehog signaling is triggered by binding of ligands with transmembrane receptor Ptch and is subsequently mediated by transcriptional effectors belonging to the Gli family, whose functions is tuned by a number of molecular interactions and post-synthetic modifications. The complex of these regulatory circuitries provides a tight control of developmental processes, mainly involving the **modulation of genes determining the fate of stem cells.** Similarly, **Hedgehog regulates cancer stem cells fostering tumorigenesis.** To this regard, these processes represent promising targets for novel therapeutic strategies aiming at the control of stemness reactivation and maintenance in cancer.

19) Kim, James, et al. "Itraconazole and arsenic trioxide inhibit Hedgehog pathway activation and tumor growth associated with acquired resistance to smoothened antagonists." Cancer cell 23.1 (2013): 23-34.
Recognition of the multiple roles of Hedgehog signaling in cancer has prompted intensive efforts to develop targeted pathway inhibitors. Leading inhibitors in clinical development act by binding to a common site within Smoothened, a critical pathway component. Acquired Smoothened mutations, including **SMOD477G**, confer resistance to these inhibitors. We report here that **itraconazole and arsenic trioxide**, two agents in clinical use that **inhibit Hedgehog signaling** by mechanisms distinct from that of current Smoothened antagonists, retain inhibitory activity in vitro in the context of all reported resistance-conferring Smoothened mutants and GLI2 overexpression. Itraconazole and arsenic trioxide, alone or in combination, inhibit the growth of medulloblastoma and basal cell carcinoma in vivo, and prolong survival of mice with intracranial drug-resistant SMOD477G medulloblastoma.

Mantle Cell: BCL-2 down regulated by Inhibiting HH pathway

20) Campbell, Victoria, and Mhairi Copland. "Hedgehog signaling in cancer stem cells: a focus on hematological cancers." Stem cells and cloning: advances and applications 8 (2015): 27.
Expression of **BCL-2 is increased in the presence of active Hh signaling** and **down-regulated upon inhibition of the pathway**.59

Components of the Hh pathway and key downstream targets (**BCL-2 and BCL-XL**) are expressed in a variety of NHL cell lines and primary tissue,59,93 with expression of the downstream targets being influenced by the Hh pathway.

Burkitt's cells underwent apoptosis in the absence of Hh signaling both in vitro and in vivo.

In another form of aggressive NHL, **mantle cell lymphoma**, a therapy-resistant murine model showed **up-regulation of the GLI transcription factors at the gene level**,97 confirming previous work showing the **GLI transcription factors to be over-expressed in mantle cell lymphoma**, both in cell lines and primary lymphoma cells, compared to normal B cells.98 Further, targeting the GLI transcription factors with antisense oligonucleotides **down-regulated BCL-2 and Cyclin D1** resulting in decreased proliferation and increased susceptibility to chemotherapy.98

The Gli proteins are the effectors of Hedgehog (Hh) signaling

hedgehog-GLI signaling mantle cell lymphoma.

21) Mol Cancer Ther. 2008 Jun;7(6):1450-60. Targeting of sonic hedgehog-GLI signaling: a potential strategy to improve therapy for mantle cell lymphoma. Hegde GV1, Munger CM, Emanuel K, Joshi AD, Greiner TC,

Weisenburger DD, Vose JM, Joshi SS. Department of Genetics, Cell Biology, and Anatomy, Center for Research in Leukemia and Lymphoma, University of Nebraska Medical Center, Omaha, NE 68198-6395, USA.

Mantle cell lymphoma (MCL) has one of the worst clinical outcomes among the B-cell lymphomas, with a median survival of only 3 to 4 years. Therefore, a better understanding of the underlying mechanisms that regulate MCL proliferation/survival is needed to develop an effective therapy. Because sonic hedgehog (Shh)-GLI signaling has been shown to be important in the proliferation and survival of several cancers, and no such information is available for MCL, this study was undertaken. Our results show that the molecules associated with Shh-GLI signaling, such as PTCH and SMO receptors, and GLI1 and GLI2 target transcription factors were **expressed in the human MCL cell lines and primary MCL cells from patients.** Perturbation of this signaling in the presence of exogenous Shh/cyclopamine significantly ($P < 0.001$) influenced the proliferation of JVM2 MCL cells. Furthermore, **down-regulation of GLI transcription factors using antisense oligonucleotides** not only resulted in significantly ($P < 0.001$) decreased proliferation of the MCL cells but also significantly ($P < 0.05$) increased their susceptibility to chemotherapeutic drug, doxorubicin. **Also, down-regulation of GLI decreased cyclin D1 and BCL2 transcript levels, which suggests that these key molecules might be regulated by GLI in MCL.** Thus, our results indicate a significant role for Shh-GLI signaling in the proliferation of MCL, and **molecular targeting of GLI** is a potential therapeutic approach to improve the treatment for MCL.

itraconazole Inhibits HH pathway in Gastric CA

22) Hu, Qiang, et al. "Itraconazole induces apoptosis and cell cycle arrest via inhibiting Hedgehog signaling in gastric cancer cells." Journal of Experimental & Clinical Cancer Research 36.1 (2017): 50.
CCK-8 assay and colony formation assay were used to assess the effects of itraconazole on proliferation of gastric cancer cells. The expression of Hh signaling components in gastric cancer cells treated with itraconazole was evaluated by reverse-transcription polymerase chain reaction, immunoblotting and dual luciferase assay. Tumor xenograft models were used to assess the inhibitory effect of itraconazole on the proliferation of gastric cancer cells in vivo.
Results **Itraconazole could remarkably inhibit the proliferation of gastric cancer cells.** When in combination with 5-FU, itraconazole significantly reduced the proliferation rate of cancer cells. Furthermore, itraconazole could regulate the G1-S transition and induce apoptosis of gastric cancer cells. Hh signaling was abnormally activated in human gastric cancer samples. **In vitro, studies showed that the expression of glioma-associated zinc finger transcription factor 1 (Gli1) was decreased at both transcriptional and translational levels after treatment with itraconazole.** Dual luciferase assay also indicated that itraconazole could inhibit the transcription of Gli1. **In vivo studies demonstrated that monotherapy with itraconazole by oral administration could inhibit the growth of xenografts,** and that itraconazole could significantly enhance the antitumor efficacy of the chemotherapeutic agent 5-FU.

Itraconazole inhibits HH pathway in Breast Cancer

23) Wang, Xiaoya, et al. "Anti-proliferation of breast cancer cells with itraconazole: Hedgehog pathway inhibition induces apoptosis and autophagic cell death." Cancer letters 385 (2017): 128-136.

Itraconazole is a common antifungal which may have promise for treating various human cancers. We report that itraconazole was cytotoxic to MCF-7 and SKBR-3 breast cancer cell lines via apoptosis by altering mitochondria membrane potential, reducing BCL-2 expression and elevating caspase-3 activity. Itraconazole also induced autophagic cell death via LC3-II expression upregulation, P62/SQSTM1 degradation, autophagosome formation and increases in autophagic puncta. Itraconazole treatment inhibited hedgehog pathway key molecular expression, such as SHH and Gli1, resulting in promotion of apoptosis and autophagy. The anti-proliferation effect

of itraconazole-induced apoptosis and autophagy via hedgehog pathway inhibition was confirmed with Gli1 inhibitor GANT61 and SHH siRNA, GANT61 and SHH siRNA synergistically enhanced cytotoxicity induced by itraconazole. A human xenograft nude mouse model corroborated the anti-breast cancer activity as evidenced by reduced tumor size, and increased tumor tissue apoptosis and autophagy. Thus, **itraconazole has a potent anti-breast cancer activity that may be improved when combined with hedgehog pathway inhibitors.**

24) Cancer Lett. 2017 Sep 28;411:136-149. Pharmacological targeting of GLI1 inhibits proliferation, tumor emboli formation and in vivo tumor growth of inflammatory breast cancer cells.Oladapo HO1, Tarpley M1, Sauer SJ2, Addo KA1, Ingram SM1, Strepay D3, Ehe BK1, Chdid L1, Trinkler M4, Roques JR4, Darr DB5, Fleming JM6, Devi GR7, Williams KP8.

Activation of the Hedgehog (Hh) pathway effector GLI1 is linked to tumorigenesis and invasiveness in a number of cancers, with targeting of GLI1 by small molecule antagonists shown to be effective. We profiled a collection of GLI antagonists possessing distinct mechanisms of action for efficacy in phenotypic models of inflammatory and non-inflammatory breast cancer (IBC and non-IBC) that we showed expressed varying levels of Hh pathway mediators. Compounds GANT61, HPI-1, and JK184 decreased cell proliferation, inhibited GLI1 mRNA expression and decreased the number of colonies formed in TN-IBC (SUM149) and TNBC (MDA-MB-231 and SUM159) cell lines. In addition, GANT61 and JK184 significantly down-regulated GLI1 targets that regulate cell cycle (cyclin D and E) and apoptosis (Bcl2). GANT61 reduced SUM149 spheroid growth and emboli formation, and in orthotopic SUM149 tumor models significantly decreased tumor growth. We successfully utilized phenotypic profiling to identify a subset of GLI1 antagonists that were prioritized for testing in in vivo models. Our results indicated that **GLI1 activation in TN-IBC as in TNBC, plays a vital role in promoting cell proliferation, motility, tumor growth, and formation of tumor emboli.**

25) Tsubamoto, Hiroshi, Takashi Sonoda, and Kayo Inoue. "Impact of itraconazole on the survival of heavily pre-treated patients with triple-negative breast cancer." Anticancer research 34.7 (2014): 3839-3844.

EIS Dr Richard Kast Inhibiting hedgehog: itraconazole

26) Kast, Richard E., et al. "Blocking epithelial-to-mesenchymal transition in glioblastoma with a sextet of repurposed drugs: the EIS regimen." Oncotarget 8.37 (2017): 60727.

Itraconazole, an old 706 Da antifungal drug, is undergoing a renaissance of interest for its anticancer effects [199, 200]. **The primary mode of anti-cancer action is inhibition of Hh signaling** [199, 200]. Hh is an important driver of GB growth [201-207]. Hh signals through intracellular transcription factor Gli [205, 206]. Gli1-driven transcription induces EMT via induction of Snail, a repressor of E-cadherin in many other cancers.

Itraconazole inhibits release of Gli1 thus keeping it sequestered in the cytoplasm [205]. GB patients with low Gli1 expression had longer overall survival [202]. The **experimental Hh signaling inhibitor cyclopamine**, or suppressing Gli1 expression by using siRNA interference led to decreased cell proliferation and enhanced apoptosis in U87 glioma cell line [208].

In preclinical studies itraconazole inhibition of Hh signaling inhibited growth of breast cancer [209], melanoma [210], and endometrial cancer [211].

Itraconazole inhibits AKT/mTOR Endometrial

27) Anticancer Res. 2017 Feb;37(2):515-519.
Itraconazole Inhibits AKT/mTOR Signaling and Proliferation in Endometrial Cancer Cells. Tsubamoto H1,2, Inoue K3, Sakata K3, Ueda T3, Takeyama R3, Shibahara H3, Sonoda T2.

Itraconazole is a common antifungal agent that has demonstrated anticancer activity in preclinical and clinical studies. This study investigated whether itraconazole exerts this effect in endometrial cancer (EC) cells.
MATERIALS AND METHODS:Cell viability was evaluated with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, and gene and protein expression were assessed by microarray analysis and immunoblotting, respectively, in five EC cell lines.
RESULTS:Itraconazole-suppressed proliferation of AN3-CA, HEC-1A and Ishikawa cells ($p<0.05$) but not of HEC-50B or SNG-II cells. Itraconazole did not suppress GLI1 or GLI2 transcription but did **inhibit the expression of mammalian target of rapamycin (mTOR) signaling components** in AN3-CA and HEC-1A cells, while **inducing that of microtubule-associated protein 1A/1B-light chain 3-II, a marker of autophagy. ATP-binding cassette transporter A1 gene was down-regulated** in Ishikawa, HEC-50B and SNG-II cells.
CONCLUSION:Itraconazole treatment suppresses the growth of EC cells by **inhibiting AKT/mTOR signalling**.\

2017 Review

28) Pounds R, Leonard S, Dawson C, Kehoe S. Repurposing itraconazole for the treatment of cancer. *Oncology Letters*. 2017;14(3):2587-2597.
Itraconazole is a broad-spectrum anti-fungal agent. An emerging body of in vivo, in vitro and clinical evidence have confirmed that it also possesses antineoplastic activities and has a synergistic action when combined with other chemotherapeutic agents. It acts via several mechanisms to prevent tumour growth, including **inhibition of the Hedgehog pathway, prevention of angiogenesis, decreased endothelial cell proliferation, cell cycle arrest and induction of auto-phagocytosis.** These allow itraconazole, either alone or in combination with other cytotoxic agents, to increase drug efficacy and overcome drug resistance.

SImultaneous m-TOR inhibition and Ibrutinib synergistic, potential therapeutic modality for the treatment of MCL.

29) Int J Cancer. 2018 Jan 1;142(1):202-213. The mTOR kinase inhibitor everolimus synergistically enhances the anti-tumor effect of the Bruton's tyrosine kinase (BTK) inhibitor PLS-123 on Mantle cell lymphoma.Li J1, Wang X1, Xie Y1, Ying Z1, Liu W1, Ping L1, Zhang C1, Pan Z2, Ding N1, Song Y1, Zhu J1.

Mantle cell lymphoma (MCL) is an aggressive and incurable malignant disease. Despite of general chemotherapy, relapse and mortality are common, highlighting the need for the development of novel targeted drugs or combination of therapeutic regimens. Recently, several drugs that target the B-cell receptor (BCR) signaling pathway, especially the **Bruton's tyrosine kinase (BTK) inhibitor ibrutinib**, have demonstrated notable therapeutic effects in relapsed/refractory patients, which indicate that pharmacological inhibition of BCR pathway holds promise in MCL treatment. Here, **we have developed a novel irreversible BTK inhibitor, PLS-123, that has more potent and selective anti-tumor activity than ibrutinib in vitro and in vivo.** Using in vitro screening, we discovered that the combination of PLS-123 and the **mammalian target of rapamycin (mTOR) inhibitor everolimus exert synergistic activity** in attenuating proliferation and motility of MCL cell lines. **Simultaneous inhibition of BTK and mTOR resulted in marked induction of apoptosis and cell cycle arrest in the G1 phase,** which were accompanied by upregulation of pro-apoptotic proteins (cleaved **Caspase-3, cleaved PARP and Bax**), repression of anti-apoptotic proteins (**Mcl-1, Bcl-xl and XIAP**), and downregulation of regulators of the G1/S phase transition (**CDK2, CDK4, CDK6 and Cyclin D1**). Gene expression profile analysis revealed simultaneous treatment with these agents led to **inhibition of the JAK2/STAT3, AKT/mTOR signaling** pathways

and SGK1 expression. Finally, the anti-tumor and pro-apoptotic activities of combination strategy have also been demonstrated using **xenograft mice models**. Taken together, **simultaneous suppression of BTK and mTOR may be indicated as a potential therapeutic modality for the treatment of MCL.**

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Addition of mTOR inhibitor reversal of drug resistance and enhanced anti-MCL activity in MCL patient samples and patient-derived xenograft models.

TME Tumor Microenvironment Adaptive Reprogramming of Kinome

30) Zhao, Xiaohong, et al. "Unification of de novo and acquired ibrutinib resistance in mantle cell lymphoma." Nature Communications 8 (2017).

The novel Bruton's tyrosine kinase inhibitor ibrutinib has demonstrated high response rates in B-cell lymphomas; however, a growing number of ibrutinib-treated patients **relapse with resistance and fulminant progression.** Using chemical proteomics and an organotypic cell-based drug screening assay, **we determine the functional role of the tumour microenvironment (TME) in ibrutinib activity and acquired ibrutinib resistance.** We demonstrate that MCL cells develop ibrutinib resistance through evolutionary processes driven by **dynamic feedback between MCL cells and TME**, **leading to kinome adaptive reprogramming, bypassing the effect of ibrutinib and reciprocal activation of PI3K-AKT-mTOR and integrin-β1 signalling**. **Combinatorial disruption of B-cell receptor signalling and PI3K-AKT-mTOR axis leads to release of MCL cells from TME, reversal of drug resistance and enhanced anti-MCL activity in MCL patient samples and patient-derived xenograft models.** This study unifies TME-mediated de novo and acquired drug resistance mechanisms and provides a novel combination therapeutic strategy against MCL and other B-cell malignancies.
Itraconazole Profoundly inhibits mTOR

Itraconazole Inhibits HH, WNT Akt in Cerv CA

31) Ueda, Tomoko, et al. "Itraconazole Modulates Hedgehog, WNT/β-catenin, as well as Akt Signalling, and Inhibits Proliferation of Cervical Cancer Cells." Anticancer Research 37.7 (2017): 3521-3526.

8-fold down-regulation in the expression of GLI1, WNT4 and WNT10A among itraconazole-treated CaSki cells. Moreover, the transcription of sterol carrier protein-2 and ATP-binding cassette transporter-1 was unaffected by itraconazole. **Immunoblots showed suppression in β-catenin expression and Akt phosphorylation.**

Itraconazole Inhibits VEGF

32) Nacev, Benjamin A., et al. "The antifungal drug itraconazole inhibits vascular endothelial growth factor receptor 2 (VEGFR2) glycosylation, trafficking, and signaling in endothelial cells." Journal of Biological Chemistry 286.51 (2011): 44045-44056.

Itraconazole is a **safe** and **widely used antifungal drug** that was recently found to possess **potent antiangiogenic** activity. Currently, there are four active clinical trials evaluating itraconazole as a cancer therapeutic. Tumor growth is dependent on angiogenesis, which is driven by the secretion of growth factors from the tumor itself. We report here that itraconazole significantly **inhibited the binding of vascular endothelial growth factor (VEGF) to VEGF receptor 2 (VEGFR2)** and that both VEGFR2 and an immediate downstream substrate, phospholipase C γ1, failed to become activated after VEGF stimulation. These effects were due to a

defect in VEGFR2 trafficking, leading to a decrease in cell surface expression, and were associated with the accumulation of immature N-glycans on VEGFR2. Small molecule inducers of lysosomal cholesterol accumulation and mammalian target of rapamycin (mTOR) inhibition, two previously reported itraconazole activities, failed to recapitulate itraconazole's effects on VEGFR2 glycosylation and signaling. Likewise, glycosylation inhibitors did not alter cholesterol trafficking or inhibit mTOR. Repletion of cellular cholesterol levels, which was known to rescue the effects of itraconazole on mTOR and cholesterol trafficking, was also able to restore VEGFR2 glycosylation and signaling. This suggests that the new effects of itraconazole occur in parallel to those previously reported but are downstream of a common target. We also demonstrated that itraconazole globally reduced poly-N-acetyllactosamine and tetra-antennary complex N-glycans in endothelial cells and induced hypoglycosylation of the epidermal growth factor receptor in a renal cell carcinoma line, suggesting that itraconazole's effects extend beyond VEGFR2.

"CD40L + Ck" coculture model

targeting BclxL – BTK inhibitor, Type II anti-CD20

33) NOVEL TARGETED STRATEGIES TO OVERCOME MICROENVIRONMENT-DEPENDENT RESISTANCE IN MANTLE CELL LYMPHOMA D. Chiron, A. Papin,C. Bellanger,M. Amiot,S. Le Gouill,C. Pellat-Deceunynck

Introduction: Mantle cell lymphoma (MCL) accumulates in lymph nodes (LN) and disseminates early on in extranodal tissues, but little attention has been paid to the importance of microenvironments in this pathology. Further investigations integrating the key role of surrounding cells are now needed to overcome drug resistance in this incurable malignancy.
Despite a significant level of the proliferation index Ki67 in LN, we did not detect any proliferating peripheral blood (PB) MCL cells, suggesting a major role of the tumor ecosystem. To determine interactions that could support survival and proliferation, primary MCL cells were cocultured in several conditions ex vivo. **In all the 21 samples tested**, **CD40L induced cell-cycle progression, which was amplified by a MCL-specific cytokine cocktail (Ck)** (Chiron et al., Blood 2016). Now, to characterize the microenvironment-dependent molecular modulations, we performed RNA-seq in MCL cells from PB or cocultured (n = 8) and compared with genes expressed in MCL cells from LN and PB (Geo, PB n = 77, LN n = 107).

More than 65% of genes induced in the "CD40L + Ck" coculture model are also upregulated in the LN compared to PB. Our model recapitulates molecular signatures that are characteristic of MCL such as cell cycle, BCR, NFkB/NIK and survival, confirming the relevance of the coculture. We further studied the coculture-induced regulation of genes belonging to the survival signature and especially the druggable Bcl2 family. We first observed that microenvironment signalings result in an unbalanced regulation of anti- and pro-apoptotic proteins in MCL, but not in normal B cells (CD19 + CD5+). The **major regulation was an increase in expression of Bcl-xL associated with a downregulation of Bim and Noxa.** Using the functional BH3-profiling assay, we demonstrated that, whereas PB MCL cells are dependent on Bcl2 for survival, Bcl-xL upregulation was responsible for loss of mitochondrial priming and resistance. Consequently, **whereas Bcl2 BH3-mimetic efficiently triggered apoptosis in PB MCL, cells protected by the microenvironment were resistant.**

We then hypothesized that **targeting BclxL** could increase treatment efficacy. Using our coculture model, we developed efficient targeted strategies (i.e., **BTK inhibitor, Type II anti-CD20**), which **counteract BclxL overexpression and overcome drug resistance** in primary cells ex vivo. This strategy should target cells protected into their niches and our ongoing Trial (NTC#02558816) will rapidly determine in vivo efficacy in MCL.

Conclusions: In summary, we reported here the development of a model that provides new insights into the microenvironment-dependent molecular regulation. Our increased understanding of intrinsic abnormalities and the integration of extrinsic signaling offer new opportunities to design mechanism-based strategies to overcome drug resistance in MCL and other B-cell malignancies.

Overcoming Venetoclax Resistance – Obinutuzumab

34) Microenvironment-dependent proliferation and mitochondrial priming loss in mantle cell lymphoma is overcome by anti-CD20

Obinutuzumab overcomes venetoclax resistance

35) Characterization and integration of mantle cell lymphoma microenvironments are determinant for the development of rational targeted therapies

36) Conference Slides Venetoclax in MCL PP SLides

37) Rationale for Testing BH3 Mimetics in In vitro Models in Lymphoma

38) Br J Haematol. 2017 Feb;176(4):583-590. Results of a phase I-II study of fenretinide and rituximab for patients with indolent B-cell lymphoma and mantle cell lymphoma. Cowan AJ1,2, Stevenson PA1, Gooley TA1, Frayo SL1, Oliveira GR3, Smith SD1,2, Green DJ1,2, Roden JE2, Pagel JM4, Wood BL1,5, Press OW1,2, Gopal AK1,2.

Fenretinide, a synthetic retinoid, induces apoptotic cell death in B-cell non-Hodgkin lymphoma (B-NHL) and acts synergistically with rituximab in preclinical models. We report results from a phase I-II study of fenretinide with rituximab for B-NHLs. Eligible diagnoses included indolent B-NHL or mantle cell lymphoma. The phase I design de-escalated from **fenretinide at 900 mg/m2 PO BID for days 1-5 of a 7-day cycle.** The phase II portion added **375 mg/m2 IV rituximab weekly on weeks 5-9 then every 3 months.** Fenretinide was continued until progression or intolerance. Thirty-two patients were treated: 7 in phase I, and 25 in phase II of the trial. No dose-limiting toxicities were observed. The phase II component utilized fenretinide 900 mg/m2 twice daily with rituximab. The most common treatment-related adverse events of grade 3 or higher were rash (n = 3) and neutropenia (n = 3). Responses were seen in 6 (24%) patients on the phase II study, with a median duration of response of 47 months (95% confidence interval, 2-56). The combination of fenretinide and rituximab was well tolerated, yielded a modest overall response rate, but with prolonged remission durations. Further study should focus on identifying the responsive subset of B-NHL.

Ibrut plus venetoclax phase III trial POSTER

39) COMBINATION IBRUTINIB (IBR) AND VENETOCLAX (VEN) FOR THE TREATMENT OF MANTLE CELL LYMPHOMA (MCL): PRIMARY ENDPOINT ASSESSMENT OF THE PHASE 2 AIM STUDY
C.S. Tam

Background: Both ibr and ven have activity in relapsed/refractory (R/R) MCL, but complete remissions (CR) are attained in <25% with either. We sought to determine the activity of the combination in an investigator-initiated, phase 2 study.

Enrolment of 24 patients (pts) with R/R (n = 23) or frontline (n = 1) MCL completed in 09/16. Pts received **4 weeks of ibr (560 mg/d),** followed by introduction of **ven (weekly ramp-up to target 400 mg/d).** The primary endpoint was CR rate at week 16, as assessed by PET/CT, BMAT, flow & molecular MRD, and endoscopy (if baseline gut involvement). Response was calculated separately with and without knowledge of the PET result by IWG criteria (Cheson JCO 2007), in order to compare with published studies (ibr, 9% CR at wk16; ven, best CR rate 21%).

Median age of pts was 68 (range, 47-81) years. For the R/R pts (n = 23), median lines of prior therapy was 2 (1-6), 48% were refractory to last treatment, and 30% had failed previous autologous SCT. As of data cutoff on Jan 11 2017, **18 pts remain on therapy, and 6 stopped treatment due to progressive disease (4), adverse event (1) or unrelated death (1)**. At week 16, ORR was 71% (**63% CR**) and 80% of complete responders were flow-cytometry negative in the marrow (sensitivity 10-3 to 10-4). Using CT without PET, the comparison responses were CR 42%, CRu 17%, PR 17% (ORR 78%). After a median follow-up of 8.3 (range 1.4-17.7) months, the **8-month estimates of PFS and OS months are 74% and 81%**. Adverse events ≥20%, irrespective of attribution, were fatigue (71%), diarrhea (67%), nausea (50%), URTI (38%), gastro-esophageal reflux (33%), neutropenia (33%), cough (25%) and bruising (21%); with the exception of neutropenia (25% grade 3-4), these were predominantly grade 1-2 in severity. Tumour lysis syndrome occurred in 2 pts with high tumour burden, leading to revision of the protocol ven starting dose from 50 mg, to 20 mg/d.

Conclusion: The combination of ibr and ven was tolerable and achieved **CR rate of 63% at week 16 in pts with MCL**. The efficacy results compare favorably with historical results, and warrant further phase III investigation.

Lenalidomide in MCL upregulates NK Killer cells

40) Activity of lenalidomide in mantle cell lymphoma can be explained by NK cell-mediated cytotoxicity. Patrick R. Hagner,

Lenalidomide is an immunomodulatory agent that has demonstrated clinical benefit for patients with relapsed or refractory mantle cell lymphoma (MCL); however, despite this observed clinical activity, the mechanism of action (MOA) of lenalidomide has not been characterized in this setting. We investigated the MOA of lenalidomide in clinical samples from patients enrolled in the CC-5013-MCL-002 trial (NCT00875667) comparing single-agent lenalidomide versus investigator's choice single-agent therapy and validated our findings in pre-clinical models of MCL. **Our results revealed a significant increase in natural killer (NK) cells relative to total lymphocytes in lenalidomide responders compared to non-responders that was associated with a trend towards prolonged progression-free survival and overall survival.** Clinical response to lenalidomide was independent of baseline tumour microenvironment expression of its molecular target, cereblon, as well as genetic mutations reported to impact clinical response to the Bruton tyrosine kinase inhibitor ibrutinib. Preclinical experiments revealed **lenalidomide enhanced NK cell-mediated cytotoxicity against MCL cells via increased lytic immunological synapse formation and secretion of granzyme B**. In contrast, lenalidomide exhibited minimal direct cytotoxic effects against MCL cells. Taken together, these data provide the first insight into the clinical activity of lenalidomide against MCL, revealing a predominately **immune-mediated** MOA.

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41) Novel chemotherapy-free combination regimen for ibrutinib-resistant mantle cell lymphoma Samer A. Srour,Hun J. Lee,Krystle Nomie,Haige Ye,Wendy Chen,Onyeka Oriabure,Jorge Romaguera,Michael L. Wang

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Synergistic inhibition of angiogenesis by artesunate and captopril

42) Evid Based Complement Alternat Med. 2013;2013:454783. Synergistic inhibition of angiogenesis by artesunate and captopril in vitro and in vivo. Krusche B1, Arend J, Efferth T.

Artesunate and captopril inhibited blood vessel formation and growth. For the first time, we demonstrated that both drugs revealed synergistic effects when combined.

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Itraconazole potent 5-LOX inhibitor

Wnt cox-2 5-lipox

43) Roos, Jessica, et al. "Regulation of tumorigenic Wnt signaling by cyclooxygenase-2, 5-lipoxygenase and their pharmacological inhibitors: A basis for novel drugs targeting cancer cells?." Pharmacology & therapeutics 157 (2016): 43-64.

non-steroidal anti-inflammatory drugs suppress Wnt signaling by targeting the pro-inflammatory enzyme 5-lipoxygenase

genetic and pharmacological inhibition of 5-lipoxygenase led to an impairment of Wnt-dependent acute and chronic myeloid leukemic stem cells.

We believe that **5-lipoxygenase inhibitors might represent a novel type of Wnt inhibitor**

(VEGF-A), a potent and well-characterized pro-angiogenic protein, is directly regulated by the TCF/ß-catenin complex

several groups have shown that the Wnt/ß-catenin signaling pathway is required for the self-renewal of LSCs derived from either HSC or more differentiated granulocyte macrophage progenitors (GMP)

the **activation of the nuclear receptors for retinoic acid and vitamin D by the respective ligands inhibits Wnt signaling**. This influence was attributed to interaction of the nuclear receptors with ß-catenin, which sequester ß-catenin away from TCF or coactivators like p300 and CBP (Takada et al., 2012).

clinical data suggest that the cancer-protective effect of NSAIDS is both due to their **COX-inhibiting potential as well as to interference with the Wnt/ß-catenin pathway, independently of COX.**

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Diclofenac and celecoxib suppress the activation of Wnt/β-catenin/Tcf signaling.

44) Neurochem Res. 2013 Nov;38(11):2313-22. Nonsteroidal anti-inflammatory drugs diclofenac and celecoxib attenuates Wnt/β-catenin/Tcf signaling pathway in human glioblastoma cells.
Sareddy GR1, Kesanakurti D, Kirti PB, Babu PP.

Glioblastoma, the most common and aggressive primary brain tumors, carry a bleak prognosis and often recur even after standard treatment modalities. Emerging evidence suggests that deregulation of the Wnt/β-catenin/Tcf signaling pathway contributes to glioblastoma progression. Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit tumor cell proliferation by suppressing Wnt/β-catenin/Tcf signaling in various human malignancies. In this study, we sought to inhibit Wnt/β-catenin/Tcf signaling in glioblastoma cells by the **NSAIDs diclofenac and celecoxib. Both diclofenac and celecoxib significantly reduced the proliferation, colony formation and migration of human glioblastoma cells**. Diclofenac and **celecoxib downregulated β-catenin/Tcf reporter activity**. Western and qRT-PCR analysis showed that diclofenac and **celecoxib reduced the expression of β-catenin target genes Axin2, cyclin D1 and c-Myc**. In addition, the **cytoplasmic accumulation and nuclear translocation of β-catenin was significantly reduced following diclofenac and celecoxib treatment.** Furthermore, diclofenac and **celecoxib significantly increased phosphorylation of β-catenin and reduced the phosphorylation of GSK3β.** These results clearly indicated that diclofenac and celecoxib are potential therapeutic agents against glioblastoma cells that act by suppressing the activation of Wnt/β-catenin/Tcf signaling.

Targeting the beta-catenin/APC pathway: effects of celecoxib

44) Maier, Thorsten Jürgen, et al. “Targeting the beta-catenin/APC pathway: a novel mechanism to explain the cyclooxygenase-2-independent anticarcinogenic effects of celecoxib in human colon carcinoma cells.” The FASEB journal 19.10 (2005): 1353-1355.Targeting beta-catenin APC anticarcinogenic effects of celecoxib in colon carcinoma Maier Thorsten Jürgen FASEB 2005

An interesting candidate is the COX-2-selective inhibitor **celecoxib, which has been shown to prevent the formation of pretumorous adenomatous polyps in patients with familial adenomatous polyposis (FAP), a disease owing to a dysfuncton of the β-catenin/ adenomatous polyposis coli**(APC) signaling pathway (1). **Currently, celecoxib is the only NSAID, which was approved by the FDA and EMEA for the treatment of these FAP patients.** The ability of celecoxib to primarily inhibit the initial stages of colorectal cancer progression prompted us to speculate that the **β-catenin/APC pathway could be a possible target of this drug.**

celecoxib treatment truly **decreased the DNA binding-activity of βcatenin/TCF/lef transcription complexes** in human Caco-2 colon cancer cells.

celecoxib-induced apoptosis in human colon carcinoma cell lines was accompanied by a **strong activation of caspase-3 and –9**

the decrease in DNA-binding activity of the β-catenin/TCF/Lef transcription complex after celecoxib treatment contributes to both, induction of cell cycle arrest by inhibiting the transcription of cell proliferation promoting genes and induction of apoptosis by transcriptional activation of caspase-3, -7, and -9.

Therefore, we put forward the hypothesis that celecoxib induces degradation of β-catenin partly by inhibition of PDK-1, which results in **inactivation of Akt kinase and in subsequent activation of GSK-3β.** This hypothesis is supported by our findings that celecoxib treatment **truly reduced GSK-3β− phosphorylation at 9Ser, the specific phosphorylation site of Akt, leading to activation of the GSK-3β enzyme.** The active GSK-3β kinase could then phosphorylate membrane-associated β-catenin, which translocates into the cytoplasm, where it is degraded by the proteasomal pathway.

we have obtained evidence that targeting the β-catenin/APC signaling pathway might be a novel approach to explain COX-2 independent anticarcinogenic effects of celecoxib

In a subsequent study, **inhibition of 5-LO** and FLAP was found to **induce apoptosis** in **MCL cell lines and primary CLL cells, suggesting an important role for 5-LO and/or its products in MCL and other B cell malignancies.** This was supported by the fact that **5-LO was up-regulated ~7-fold in MCL cells** compared to normal B cells (Boyd et al., 2009). In addition, Runarsson et al. observed that treatment of B-CLL cells with the iron-ligand 5-LO inhibitor BWA4C and FLAP inhibitor MK886 **counteracted CD40-dependent activation of these cells by inhibiting CD40-induced DNA synthesis and CD40-induced expression of CD23, CD54, and CD150.** This finding was likely LT-dependent, since addition of exogenous LTB4 almost completely reversed the observed effects (Runarsson et al., 2005).

5-LO overexpressed in MCL cells
inhibitors promising therapeutic strategy for MCL and CLL.

45) Boyd, Robert S., et al. "Protein profiling of plasma membranes defines aberrant signaling pathways in mantle cell lymphoma." Molecular & Cellular Proteomics 8.7 (2009): 1501-1515.
However, **5-lipoxygenase (5-LO), a key enzyme in leukotriene biosynthesis, was associated with lipid rafts and was up-regulated ~7-fold in MCL compared with normal B cells**. Significantly inhibitors of 5-LO activity (AA861) and 5-LO-activating protein (FLAP) (MK886, its activating enzyme) **induced apoptosis in MCL cell lines** and primary chronic lymphocytic leukemia cells, indicating an important role for the leukotriene biosynthetic pathway in MCL and other B cell malignancies.

one protein, 5-lipoxygenase (5-LO), was markedly overexpressed in MCL cells and cell lines. **Significantly inhibitors of 5-LO and the leukotriene biosynthesis pathway are potent inducers of apoptotic cell death of malignant B cells, suggesting a new therapeutic approach.**

Aberrant expression of 5-LO has been observed in a variety of cancer cells (for reviews, see Refs. 40–42) and appears to promote cell proliferation while suppressing apoptosis. 5-LO overexpression has been correlated with increased resistance to apoptosis of Epstein-Barr virus-infected lymphomas (43), and 5-LO inhibitors induce apoptosis that can be antagonized by 5-LO metabolites (i.e. 5-HETE and 15-HETE).

Our finding that 5-LO is overexpressed in MCL cells and the susceptibility of MCL cell lines and primary CLL cells to 5-LO and FLAP inhibitors indicate that this could be a promising therapeutic strategy for MCL and CLL.

46) Mahshid, Yilmaz, et al. "High expression of 5-lipoxygenase in normal and malignant mantle zone B lymphocytes." BMC immunology 10.1 (2009): 2.

Sulindac Suppresses 5-LOX

47) Steinbrink, Svenja D., et al. "Sulindac sulfide suppresses 5-lipoxygenase at clinically relevant concentrations." Cellular and molecular life sciences 67.5 (2010): 797-806.
Sulindac is a non-selective inhibitor of cyclooxygenases (COX) used to treat inflammation and pain. Additionally, non-COX targets may account for the drug's chemo-preventive efficacy against colorectal cancer and reduced gastrointestinal toxicity. Here, we demonstrate that the pharmacologically active metabolite of sulindac, sulindac sulfide (SSi), targets 5-lipoxygenase (5-LO), the key enzyme in the biosynthesis of proinflammatory leukotrienes (LTs). SSi inhibited 5-LO in ionophore A23187- and LPS/fMLP-stimulated human polymorphonuclear leukocytes (IC(50) approximately 8-10 microM). Importantly, SSi efficiently suppressed 5-LO in human whole blood at clinically relevant plasma levels (IC(50) = 18.7 microM). SSi was 5-LO-selective as no inhibition of related lipoxygenases (12-LO, 15-LO) was observed. The sulindac prodrug and the other metabolite, sulindac sulfone

(SSo), failed to inhibit 5-LO. Mechanistic analysis demonstrated that SSi directly suppresses 5-LO with an IC(50) of 20 muM. Together, these findings may provide a novel molecular basis to explain the COX-independent pharmacological effects of sulindac under therapy.

Itraconazole and clarithromycin inhibits P-gp

48) Int Forum Allergy Rhinol. 2015 Jun;5(6):477-80. doi: 10.1002/alr.21454. Epub 2015 Apr 23. Itraconazole and clarithromycin **inhibit P-glycoprotein** activity in primary human sinonasal epithelial cells.
Lam A1, Hoang JD2, Singleton A2, Han X2, Bleier BS1.

Itraconazole and clarithromycin are clinically effective in the treatment of chronic rhinosinusitis (CRS) through incompletely understood anti-inflammatory properties. P-glycoprotein (P-gp) is overexpressed in CRS and inhibition results in decreased inflammatory cytokine secretion. Both itraconazole and clarithromycin have also been shown to have P-gp inhibitory properties in other tissues, suggesting a novel explanation for their immunomodulatory effects in CRS. The purpose of this study is to therefore confirm whether these drugs are capable of inhibiting P-gp specifically in sinonasal epithelial cells.
METHODS: This was an institutional review board (IRB)-approved study in which primary sinonasal epithelial cells were cultured in 96-well plates. A Calcein AM assay was used to quantify P-gp inhibition as determined by an increase in intracellular fluorescence. A dose-response curve was generated for itraconazole and clarithromycin (maximal concentration 100 μM) and compared to that of Zosuquidar, a highly specific known P-gp inhibitor. Results were compared using a Student t test with a significance defined as $p < 0.05$.
RESULTS: Both itraconazole and clarithromycin demonstrated a dose-response curve for P-gp inhibition similar to that of Zosuquidar. The respective maximal inhibitory concentrations of Zosuquidar, itraconazole, and clarithromycin prior to induction of cytotoxicity were 0.31, 3.13, and 1.56 μM, respectively, as demonstrated by a statistically significant increase in total intracellular fluorescence ($p < 0.05$ in all groups).
CONCLUSION: Both itraconazole and clarithromycin are capable of inhibiting sinonasal epithelial cell associated P-gp. The anti-inflammatory effects of these agents in CRS may be attributable, in part, to their heretofore unrecognized P-gp modulatory properties.

49) Liang, Guanzhao, et al. "Itraconazole exerts its anti-melanoma effect by suppressing Hedgehog, Wnt, and PI3K/mTOR signaling pathways." Oncotarget 8.17 (2017): 28510.

we demonstrate that itraconazole significantly **down-regulates Gli-1, Gli-2, Wnt3A, β-catenin and cyclin D1, while it up-regulates Gli-3 and Axin-1,** indicating **potent inhibitory effects of itraconazole on Hedgehog (Hh) and Wnt signaling pathways.**

the effects of itraconazole on melanoma tumor growth in mouse and in vitro cell proliferation are assessed and the influences of itraconazole on cell developmental signaling pathways are investigated. We find that **itraconazole effectively inhibits melanoma by suppressing Hh, Wnt and PI3K-mTOR signaling pathways.**

We find that **Wnt3A (Wnt growth factor protein) declines dramatically** in SK-MEL-28 cells when treated with 1 and 2 μM itraconazole and is barely detectable when the dose is raised to 4 μM.

our results show that itraconazole treatment is able to **inhibit Ki-67** in a dose-dependent manner

itraconazole may act as a dual inhibitor for the PI3K-mTOR pathway in melanoma cells.

(i.e., 400 mg/day orally for 15 days) in patients with fungal infections, but are similar to itraconazole doses in patients with severe fungal infections [60, 61], In these cases, a high dose of itraconazole ranging from 600 to 900 mg/day can be given to patients for 3 to 16 months with close monitoring for any toxicity of this compound.

50) Inflammation Khosrow Kashfi (City University of New York School of Medicine, USA) Development of NOSH-NSAIDs: a new class of anti-inflammatory pharmaceuticals for the treatment of cancer

51) Zhang, Han, et al. "Hedgehog inhibitors selectively target cell migration and adhesion of mantle cell lymphoma in bone marrow microenvironment." Oncotarget 7.12 (2016): 14350.

The clinical benefits of a Hedgehog (Hh) inhibitor, LDE225 (NPV-LDE-225, Erismodegib), have been unclear in hematological cancers. Here, we report that LDE225 selectively inhibited migration and adhesion of mantle cell lymphoma (MCL) to bone marrows via very late antigen-4 (VLA-4) mediated **inactivation of focal adhesion kinase (FAK) signaling**. LDE225 treatment not only affected MCL cells, but also modulated stromal cells within the bone marrow microenvironment by **decreasing their production of SDF-1, IL-6 and VCAM-1, the ligand for VLA-4**. Surprisingly, LDE225 treatment alone did not suppress cell proliferation due to increased CXCR4 expression mediated by reactive oxygen species (ROS). The increased ROS/CXCR4 further **stimulated autophagy formation**. The **combination of LDE225 with the autophagy inhibitors further enhanced MCL cell death.** Our data, for the first time, revealed LDE225 selectively targets MCL cells migration and adhesion to bone marrows. The ineffectiveness of LDE225 in MCL is due to autophagy formation, which in turn increases cell viability. **Inhibiting autophagy will be an effective adjuvant therapy for LDE225 in MCL, especially for advanced MCL patients with bone marrow involvement.**

52) Wahid, Mohd, et al. "Vismodegib, itraconazole and sonidegib as hedgehog pathway inhibitors and their relative competencies in the treatment of basal cell carcinomas." Critical reviews in oncology/hematology 98 (2016): 235-241.

2016 Itraconazole for B Cell Lymphoma- Disruption of HKII from mitochondria

53) Itraconazole, an Oral Antifungal Drug, Is Active in Chemotherapy Resistant B-Cell Non-Hodgkin Lymphoma and Enhances the Anti-Tumor Activity of Chemotherapy Agents
Juan J Gu, Lianjuan Yang, Cory Mavis, Matthew J. Barth and Francisco J. Hernandez-Ilizaliturri Blood **2016** 128:5138;

ackground: Relapsed/refractory diffuse large B-cell lymphoma (DLBCL) patients previously treated with rituximab-based therapy have poor clinical outcome, according to the results from collaborative trial in relapsed aggressive lymphoma (CORAL) study. It stresses the need to identify and/or optimize novel targeted agents. To better understand the molecular mechanisms underlining the acquired resistance to rituximab, we generated and characterized several rituximab-resistant DLBCL cell lines (RRCLs). **Itraconazole, an oral antifungal agent, was reported had novel anticancer activity in basal cell carcinoma, non-small cell lung cancer and prostate cancer.** In our current work, we define and characterize the anticancer activity of itraconazole in preclinical rituximab-sensitive or -resistant lymphoma models.

Methods: A panel of rituximab-sensitive (RSCL) and rituximab-resistant (RRCL) cell lines were exposed to escalating doses of itraconazole **(0-20μM)** for 24, 48 and 72h. Changes in cell viability and cell cycle distribution were evaluated using the Presto Blue assay and flow cytometry respectively. IC50 was calculated by Graphpad Prism6 software. **Loss of mitochondrial membrane potential** ($\Delta\psi m$) following itraconazole exposure was assessed by DiOC6 and flow cytometry. Subsequently lymphoma cells were exposed to itraconazole or vehicle

and various chemotherapy agents such as doxorubicin (1μM), dexamethasone (1μM), cDDP (20μg/ml), bortezomib (20nM), carfilzomib (20nM) or MLN2238 (20nM) for 48 hours. Coefficient of synergy was calculated using the CalcuSyn software. Changes in hexokinase II (HKII), Voltage dependent anion channel protein (VDAC), LC3 and BCL-xL expression levels were determined by western blotting after exposure cells to itraconazole. VDAC-HKII interactions following in vitro exposure to itraconazole were determined by immunoprecipitation of VDAC and probing for HKII in RSCL and RRCLs.

Result: **Itraconazole consistently showed potent, specific, dose-and time- dependent inhibition of all our sensitive and resistant lymphoma cell lines.** In vitro exposure cells to itraconazole resulted in a **loss of mitochondrial membrane potential and caused G2 cell cycle arrest.** Itraconazole significantly had a synergistic anti-tumor effect combined with various chemotherapeutic agents, including doxorubicin, dexamethasone, cisplatin and different generations of proteasome inhibitors (bortezomib, carfilzomib or ixazomib) in both RSCL and RRCL. Western blot and immunoprecipitation studies demonstrated that following exposure to itraconazole, **HKII bound less to mitochondrial specific protein VDAC**. Complete silencing of HKII (using HKII siRNA interference) resulted in a rescue of loss in the mitochondrial membrane potential induced by intraconazole.

Conclusion: Taking together, our data suggest that itraconazole had a potent anti-tumor activity against rituximab-sensitive or resistant pre-clinical models. The disruption of HKII from mitochondria following itraconazole exposure may contribute to lower the mitochondrial membrane potential and enhance the chemotherapeutic efficacy. Our finding highlights **itraconazole as a potential therapeutic agent in the treatment of B-cell malignancies, and strongly supports clinical translation of its use.**

HedgeHog Pathway Upregulated in Skin Cancers

54) Li, Chengxin, Sumin Chi, and Jingwu Xie. "Hedgehog signaling in skin cancers." Cellular signalling 23.8 (2011): 1235-1243.

An increasing progress on the role of Hedgehog (Hh) signaling for carcinogenesis has been achieved since the link of Hh pathway to human cancer was firstly established. In particular, the critical role of Hh signaling in the development of Basal cell carcinoma (BCC) has been convincingly demonstrated by genetic mutation analyses, mouse models of BCCs, and successful clinical trials of BCCs using Hh signaling inhibitors. In addition, the Hh pathway activity is also reported to be involved in the **pathogenesis of Squamous Cell Carcinoma (SCC)**, melanoma and Merkel Cell Carcinoma. These findings have significant new paradigm on Hh signaling transduction, its mechanisms in skin cancer and even therapeutic approaches for BCC. In this review, we will summarize the major advances in the understanding of Hh signaling transduction, the roles of Hh signaling in skin cancer development, and the current implications of "mechanism-based" therapeutic strategies.

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55) Schneider, Sven, et al. "Expression of the Sonic hedgehog pathway in squamous cell carcinoma of the skin and the mucosa of the head and neck." Head & neck 33.2 (2011): 244-250.

Activation of the hedgehog pathway may contribute to carcinogenesis. This study characterizes the expression pattern in squamous cell carcinoma of the skin and the head and neck.
METHODS:Tissue microarrays were constructed with samples of squamous cell carcinoma of the skin and the head and neck. All tissue samples were immunohistochemically stained for 7 Hedgehog pathway molecules.
RESULTS: **Significant ($p < .0001$) overexpression of all evaluated molecules could be observed in the tumor samples compared with healthy control tissues.** Expression of Gli-2 showed significant upregulation

and that of Smoothened and Patched significant downregulation in head and neck compared with skin carcinoma. **High expression of Sonic hedgehog correlates significantly (p = .001) with poor overall survival in patients with head and neck cancer.**
CONCLUSIONS:Hedgehog signaling is differentially regulated in squamous cell carcinomas of the skin and the head and neck. Sonic hedgehog expression may serve as a prognostic factor in patients with head and neck cancer.

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Mebendazole HH Inhibitor

57) Larsen, Andrew R., et al. "Repurposing the antihelmintic mebendazole as a hedgehog inhibitor." Molecular cancer therapeutics 14.1 (2015): 3-13.

58) Popović, Dušica J., et al. "Application of a widely-used tropical anti-worm agent, mebendazole, in modern oncology." Tropical Journal of Pharmaceutical Research 16.10 (2017): 2555-2562. Application of mebendazole in modern oncology Popović Dušica Tropical J Pharm Res 2017

Anti-Parasitic Drug Vermox (mebendazole)

59) mebendazole metastatic colon cancer Nygren Acta Oncologica 2014
Nygren, Peter, and Rolf Larsson. "Drug repositioning from bench to bedside: Tumour remission by the antihelmintic drug mebendazole in refractory metastatic colon cancer." Acta Oncologica 53.3 (2014): 427-428.

60) http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4096024/
Pantziarka, Pan et al. "Repurposing Drugs in Oncology (ReDO)—mebendazole as an Anti-Cancer Agent." ecancermedicalscience 8 (2014): 443. PMC. Web. 13 Jan. 2015.

61)http://mcr.aacrjournals.org/content/6/8/1308.full
Doudican, Nicole, et al. "Mebendazole induces apoptosis via Bcl-2 inactivation in chemoresistant melanoma cells." *Molecular Cancer Research* 6.8 (2008): 1308-1315.

62) http://neuro-oncology.oxfordjournals.org/content/early/2011/07/14/neuonc.nor077.full Bai, Ren-Yuan, et al. "Antiparasitic mebendazole shows survival benefit in 2 preclinical models of glioblastoma multiforme." *Neuro-oncology* (2011): nor077.

63)Mebendazole metastatic adrenocortical carcinoma Dobrosotskaya Endocrine practice 2011 Dobrosotskaya, I. Y., et al. "Mebendazole monotherapy and long-term disease control in metastatic adrenocortical carcinoma." *Endocrine practice: official journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists* 17.3 (2011): e59.

64) Gamble, Alex, et al. "ATPS-83REPURPOSING MEBENDAZOLE AS A REPLACEMENT FOR VINCRISTINE FOR THE TREATMENT OF BRAIN TUMORS." Neuro-Oncology 17.suppl 5 (2015): v36-v36.
We found that mebendazole showed a 61% increase in animal survival time, whereas vincristine failed to show any efficacy. However, we did observe significant neuropathy (as measured by sensory allodynia) induced by

mebendazole treatment, similar to that caused by vincristine. In conclusion, our results strongly support the clinical use of mebendazole as a replacement for vincristine for the treatment of brain tumors.

65) Bodhinayake, Imithri, Marc Symons, and John A. Boockvar. “Repurposing Mebendazole for the Treatment of Medulloblastoma.” Neurosurgery 76.2 (2015): N15-N16. Repurposing Mebendazole Treatment Medulloblastoma Neurosurgery Bodhinayake 2015

Good Photos of Skin Cancer Regression !!!

66) Kim, Daniel J., et al. “Open-label, exploratory phase II trial of oral itraconazole for the treatment of basal cell carcinoma.” J Clin Oncol 32.8 (2014): 745-751. Phase II trial oral itraconazole for basal cell carcinoma Kim Daniel J Clin Oncol 2014

Itraconazole, a US Food and Drug Administration-approved antifungal drug, inhibits the Hedgehog (HH) signaling pathway, a crucial driver of basal cell carcinoma (BCC) tumorigenesis, and reduces BCC growth in mice. We assessed the effect of itraconazole on the HH pathway and on tumor size in human BCC tumors.
PATIENTS AND METHODS:

Patients with ≥ one BCC tumor > 4 mm in diameter were enrolled onto two cohorts to receive oral itraconazole 200 mg twice per day for 1 month (cohort A) or 100 mg twice per day for an average of 2.3 months (cohort B). The primary end point was change in biomarkers: Ki67 tumor proliferation and HH activity (GLI1 mRNA). Secondary end points included change in tumor size in a subset of patients with multiple tumors.
RESULTS:

A total of 29 patients were enrolled, of whom 19 were treated with itraconazole. Itraconazole treatment was associated with two adverse events (grade 2 fatigue and grade 4 congestive heart failure). Itraconazole reduced cell proliferation by 45% (P = .04), HH pathway activity by 65% (P = .03), and reduced tumor area by 24% (95% CI, 18.2% to 30.0%). Of eight patients with multiple nonbiopsied tumors, four achieved partial response, and four had stable disease. Tumors from untreated control patients and from those previously treated with vismodegib showed no significant changes in proliferation or tumor size.
CONCLUSION:

Itraconazole has anti-BCC activity in humans. These results provide the basis for larger trials of longer duration to measure the clinical efficacy of itraconazole, especially relative to other HH pathway inhibitors.

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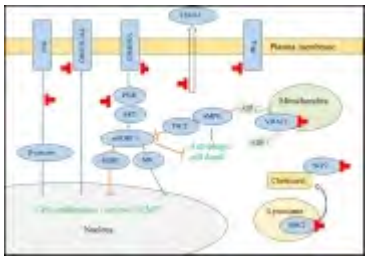

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Itraconazole Anti-Cancer Anti-Fungal Drug

Description

The anti-fungal drug Itraconazole is a Repurposed Anti-Cancer Drug

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