Defining structure activity relationships for GPCR engagement and anti-cancer efficacy of imipridone small molecules

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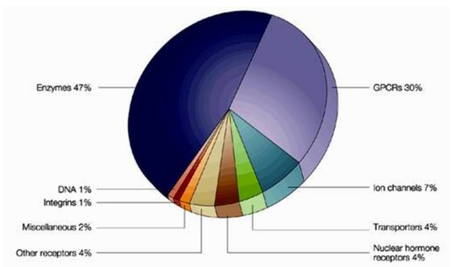
Abstract

G protein-coupled receptors (GPCRs) represent the most widely exploited superfamily of drug targets for FDA-approved therapies for many diseases, however, these receptors are underexploited for oncology. ONC201 is a selective antagonist of GPCRs dopamine receptor D2 (DRD2) and DRD3 that has been shown to induce tumor regressions with a benign safety profile in high grade glioma patients. ONC201 (benzyl-2-methylbenzyl-imipridone) is the founding member of the imipridone class of small molecules that share a unique tri-heterocyclic core chemical structure. Imipridones share several chemical and biological properties that are desirable drug-like characteristics: oral administration, wide therapeutic window, chemical stability and blood brain barrier penetrance. In this study, we profiled a series of imipridones for GPCR engagement and anti-cancer efficacy. Several imipridones were screened against a large panel of human GPCRs using a β-arrestin recruitment assay. The imipridones tested resulted in GPCR agonist/antagonist activity (threshold set at >20% activity) that was heterogenous, but exclusive among Class A GPCRs that represent the largest class. Minor chemical modifications to the ONC201 chemical structure caused large shifts in agonist versus antagonist activity and selectivity for GPCRs. Specifically, switching the ONC201 imipridone core from an angular to a linear isomer resulted in loss of DRD2 antagonist activity and impaired inhibition of cancer cell viability, indicating the imipridone core structure is critical for GPCR engagement and anti-cancer effects. The addition of electron withdrawing groups (e.g. di- or tri-halogen substitution) to the methyl benzyl ring improved potency for GPCR engagement and anti-cancer effects, but not for the benzyl ring. Loss of the benzyl ring impaired anti-cancer effects. Among all of the GPCR hits identified, maximal variance in imipridone GPCR engagement was identified for DRD2/DRD3 antagonism and GPR132 agonism that were prioritized considering their known biological relevance in oncology. ONC206 (benzyl-2,4-difluoromethylbenzyl-imipridone) emerged as the most selective and potent antagonist for D2-like dopamine receptors that are overexpressed and critical for survival in several cancers. ONC212 (benzyl-4-trifluoromethylbenzyl-imipridone) was the most selective and potent agonist for tumor suppressor GPR132. Both compounds were tested in the GDSC panel of >1000 cancer cell lines and demonstrated broad spectrum nanomolar inhibition of cancer cell viability and a wide therapeutic window. GPCR target expression correlated with anti-cancer efficacy in the GDSC panel for both compounds, providing potential biomarkers of response. Thus, chemical derivatization of ONC201 has generated a class of novel GPCR-targeting agents with promising preclinical efficacy and safety profiles in oncology.

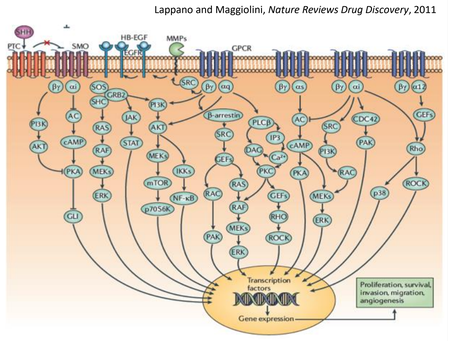
Background

GPCRs historically underexploited in oncology

- Thirty to fifty percent of marketed drugs are estimated to exert their clinical effects via GPCRs and out of a total of 219 new molecular entities (NMEs) approved by the US Food and Drug Administration (FDA) from 2005 to 2014, 54 (25 percent) target GPCRs.
- GPCRs hijacked by cancers through overexpression of the receptor or mutation/overexpression of key signaling mediators
- Control an array of pro-survival signaling (e.g. Ras) and alleviate stress pathways (e.g. integrated stress response) in cancer cells
- Aberrant expression of GPCRs is highly prevalent across all tumor types establishing GCPRs as a significant therapeutic opportunity in oncology
- GPCRs dysregulated but underexploited in oncology



Lappano and Maggiolini, *Nature Reviews Drug Discovery*, 2011



Hopkins and Groom, *Nature Reviews Drug Discovery*, 2012

Imipridones: New Class of GPCR-Targeting Therapies

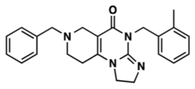
- Unique tri-heterocyclic core structure
- Possess attractive chemical and biological properties
  - Orally active
  - Wide therapeutic window
  - Chemically stable
  - Crosses BBB
- Core structure enabled development of a portfolio of compounds that selectively target distinct GPCRs

Disclosures: VVP, ARK, MS, WO, and JEA are employees and/or shareholders of Oncoceutics. NSM is a previous consultant of Oncoceutics

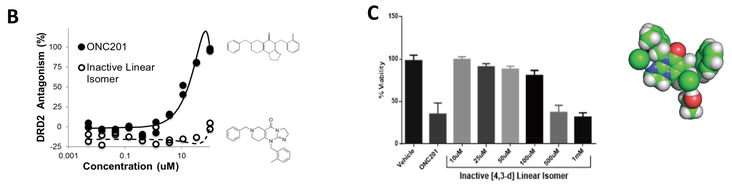
Results

ONC201: First-in-class imipridone small molecule with novel pharmacophore

- ONC201 tri-heterocyclic angular core structure critical for GPCR engagement and efficacy as isomer with linear core structure was inactive.



Molecular Formula: $C_{24}H_{26}N_4O$
Molecular Weight: 386.5

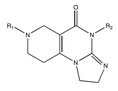


Wagner et al. *Oncotarget* 2015

Figure 1. (A) ONC201 structure (B) ONC201 and its linear isomer were tested in (B) DRD2 antagonism and (C) cell viability assays

Imipridone SAR: GPCR Engagement

- Imipridones were screened against a large panel of GPCRs in B-arrestin assay
- Minor chemical changes cause dramatic shifts:
  - Modality: agonist vs antagonist
  - Selectivity



Imipridone	R1	R2
ONC201	CH_2Ph	CH_2-((2-CH_3)-Ph)
ONC206	CH_2Ph	CH_2-(2,4-di F-Ph)
ONC207	H	CH_2-((2-CH_3)-Ph)
ONC212	CH_2Ph	CH_2-(4-CF_3-Ph)
ONC213	CH_2Ph	CH_2-(3,4-di F-Ph)
ONC234	3,4-diClPhCH2	CH_2-(4-CF_3-Ph)
ONC236	CH2-3-thienyl	CH_2-(4-CF_3-Ph)
ONC237	CH2CH(OH)Ph	CH_2-(4-CF_3-Ph)

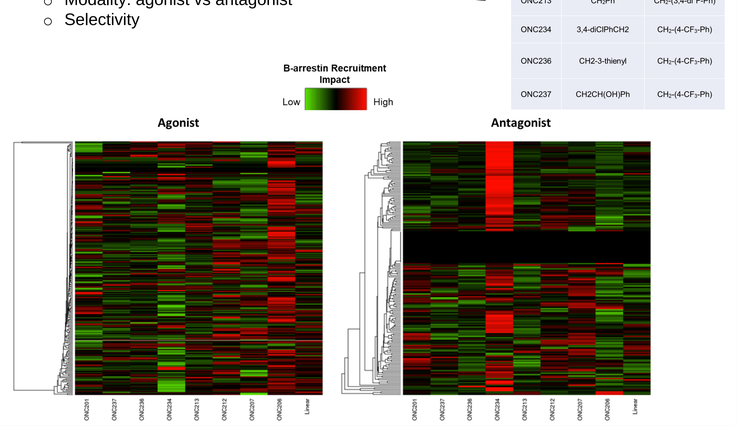
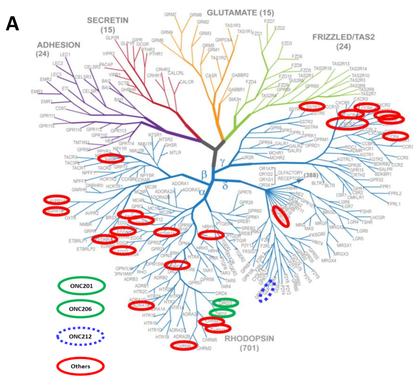
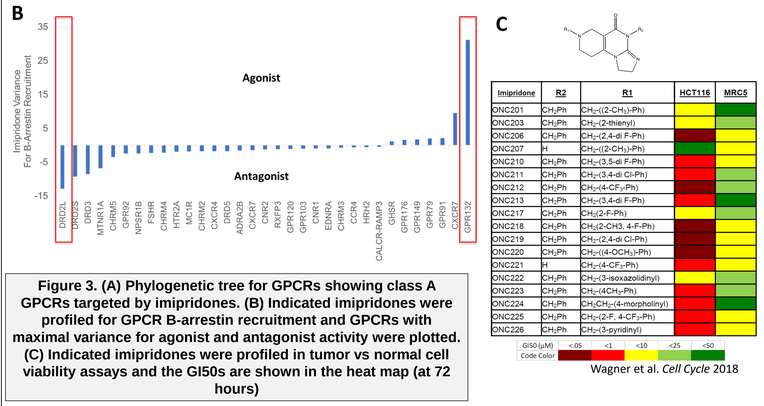


Figure 2. The indicated imipridone small molecules (10uM) were screened in a large panel (>150) GPCRs using the Eurofins DiscoverX B-arrestin recruitment assay.

Imipridone SAR: Class A GPCR Engagement and anti-cancer efficacy

- Imipridone scaffold selective for Class A GPCRs
  - Largest class with over 700 members
- Maximal variance in imipridone GPCR engagement identified for DRD2 and GPR132 allowing determination of structure activity relationship
- Portfolio prioritized for these targets considering relevance in oncology
- Imipridones screened for cell viability effects on tumor and normal cells
- ONC206, ONC212 identified as promising candidates based on
  - anti-cancer potency
  - therapeutic window



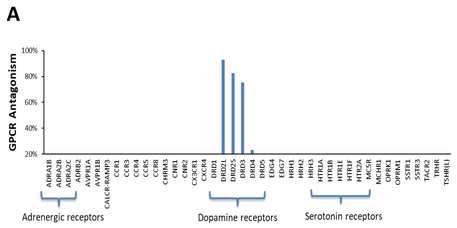


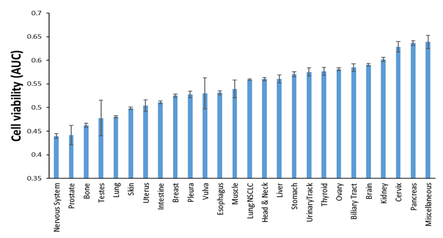
Wagner et al. *Cell Cycle* 2018

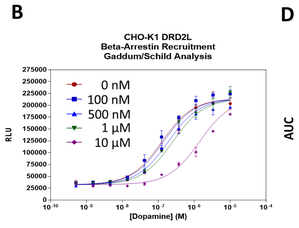
Figure 3. (A) Phylogenetic tree for GPCRs showing class A GPCRs targeted by imipridones. (B) Indicated imipridones were profiled for GPCR B-arrestin recruitment and GPCRs with maximal variance for agonist and antagonist activity were plotted. (C) Indicated imipridones were profiled in tumor vs normal cell viability assays and the GI50s are shown in the heat map (at 72 hours)

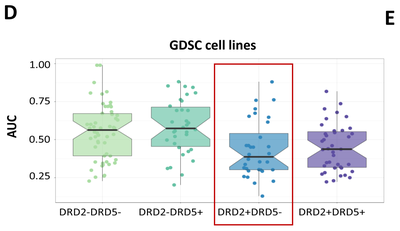
ONC206: Differentiated DRD2/3 Antagonist for Dopamine Dysregulated Tumors

- Unique receptor pharmacology enables ten fold improvement in DRD2 antagonism relative to ONC201 and distinct spectrum of activity
- Low nanomolar potency in >1000 GDSC cancer cell lines
- DRD2 dysregulated tumors include neuro-oncology tumors as most sensitive
- DRD2+/DRD5- expression signature in >1000 GDSC cancer cell lines predicts innate ONC206 sensitivity









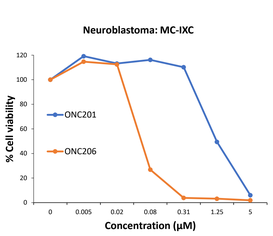


Figure 4. (A) ONC206 (10uM) was screened in a large panel of GPCRs using the Eurofins DiscoverX B-arrestin recruitment assay. (B) Schild analysis for ONC206 DRD2 antagonism in the Eurofins DiscoverX B-arrestin recruitment assay (C) In vitro sensitivity of >1000 Genomics of Drug Sensitivity in Cancer (GDSC) human cancer cell lines to ONC206 (72h) averaged and organized by tumor type. (D) Dopamine receptor expression (DRD2/DRD5) predicts ONC206 efficacy in the GDSC cell lines (E) Cell viability curve for ONC201 and ONC206 in neuroblastoma cells

ONC212:The First GPR132-Targeting Compound For Leukemia

- ONC212 is a highly selective agonist of orphan GPCR tumor suppressor GPR132 at nanomolar concentrations
- Nanomolar in vitro anti-cancer activity in >1000 GDSC cancer lines, where Leukemia is the most sensitive.
- GPR132 expression in >1000 cancer cell lines predicts innate ONC212 sensitivity

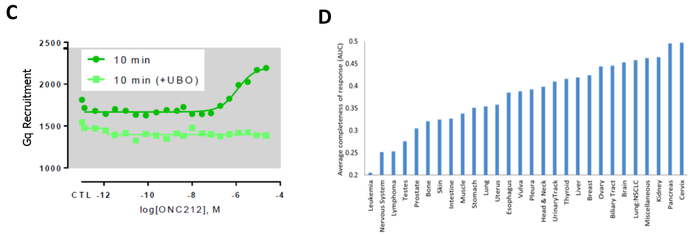


Figure 5. (A) ONC212 was screened in a large panel of GPCRs using the Eurofins DiscoverX B-arrestin recruitment assay. (B) Multi-dose validation of ONC212 GPR132 agonism in the Eurofins DiscoverX B-arrestin recruitment assay. (C) GPR132 Gq recruitment in response to ONC212 +/- Gq inhibitor (UBO) (D) In vitro sensitivity of >1000 Genomics of Drug Sensitivity in Cancer (GDSC) human cancer cell lines to ONC212 (72h) averaged and organized by tumor type. (E) GPR132 expression predicts ONC212 efficacy in the GDSC cell lines.

Conclusion

- Imipridone small molecules act as antagonists/agonists for Class A GPCRs that represent an underexploited therapeutic opportunity in oncology.
- Switching the imipridone core from an angular to a linear isomer resulted in loss of GPCR engagement and anti-cancer efficacy, while the addition of electron withdrawing groups to the methyl benzyl ring improved the potency for both effects.
- Maximal variance for imipridone engagement was observed for DRD2 and GPR132.
- ONC206 emerged as the most potent and selective DRD2/3 antagonist. Dopamine receptor expression correlated with inhibition of cell viability.
- ONC212 is the first selective agonist for tumor suppressor GPR132 in oncology. GPR132 expression predicts ONC212 efficacy.