The gamma secretase inhibitor AL101 combined with other drugs for dual targeting of Notch dysregulated tumors

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Background

Adenoid Cystic Carcinoma (ACC) & Triple Negative Breast Cancer (TNBC) are tumors associated with Notch dysregulation. Ayala is targeting Notch activated tumors with investigational small molecule pan-Notch γ-secretase inhibitors (GSIs) AL101 & AL102. AL101 is currently being investigated in 2 Phase II open-label, single-arm, multicenter studies in ACC (ACCURACY; NCT03691207) and TNBC (TENACITY; NCT04461600) patients harboring known Notch1-4 activating alterations.

A comprehensive analysis of the genetic hallmarks of recurrent/metastatic ACC (Ho et al, 2019) demonstrated enrichment for alterations in key Notch and chromatin-remodeling genes.

The Notch pathway may interact via crosstalk with additional pathways in ACC, and here we describe their identification using a bioinformatic approach.

Methods

For the bioinformatics, gene expression from 3 independent publications that compared ACC tumors to normal samples was analyzed. Normalization and differentially expressed genes were calculated using R packages DESeq2 and RMA + Limma for RNASeq and microarray experiments, respectively. Differential expression was calculated for the ACC samples versus normal for each study. The most significant differentially expressed genes (abs(logFC)>1, FDR<0.05) from each study were intersected and only genes with the same directionality were selected. Pathway enrichment was performed using MetaCore™ (Clarivate), and we identified significance (p-value<0.05).

Using Jaccard index we clustered the pathways that had overlapping differentially expressed genes to create independent pathways. Our strategy for compound selection focused on those pathways that are targetable with approved oncology drugs.

To test whether the combinations provided added benefit, we compared each drug alone or their combination using PDX models.

Acknowledgements

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Conflicts of Interest

RR, TL, AS, EH, RW & JK are employees of Ayala Pharmaceuticals. RF & AH are both Investigators in the ACCURACY study and receive consultancy fees from Ayala.

Result 1: Bioinformatic Identification of Upregulated Pathways in ACC

Gene expression data from:
[1]Gao et al; 12 ACC vs 12 Normal
[2]Bell et al, 39 ACC vs 4 Normal
[3]Andersson et al; 13 ACC vs 7 Normal

→ Significant Differentially Expressed (DE) genes in each study

↓ abs(log2FC) >1 & FDR<0.05, reads>50

Common significantly DE genes (n=614)

↓ Pathway Enrichment Analysis
MetaCore™ (Clarivate) FDR<0.05

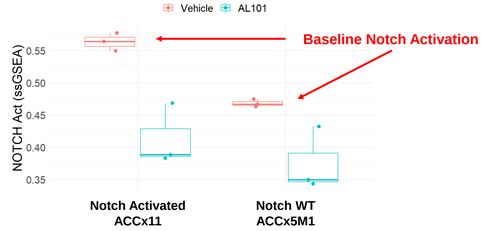
71 Dysregulated Pathway Maps

↓ Combine maps with common genes

27 Individual Pathways → Ranked by Significance:
Notch
WNT/beta-catenin
Cell cycle (CDK6)
Apoptosis (Bcl2)
FGFR signaling
ECM remodeling
Cell adhesion
EMT

[1]doi: 10.18632/oncotarget.2985
[2]doi: 10.1002/cncr.29959
[3]doi: 10.1093/jnci/djx017

Result 2: AL101 Inhibits Notch Signaling in Both Activated & WT ACC PDX



This data suggests combinations with GSI is possible in any ACC

Figure 1: AL101 treatment inhibits Notch target gene expression in both Notch activated (ACCx11) & WT (ACCx5M1) PDX Tumors

Single sample gene-set enrichment (ssGSEA; (Barbie et al, doi: 10.1038/nature08460) to determine expression levels for a set of 21 manually curated NOTCH target genes. The basal Notch target gene expression is higher in the activated ACCx11 tumor compared to WT ACCx5M1. However, after treatment with AL101, the ssGSEA is reduced in both tumors.

Selection of Compounds for In Vivo Combo Studies

Pathway	Rationale	Tool Compound	Dose & Regimen
Notch	Genetic hallmarks of recurrent/metastatic ACC demonstrated enrichment for alterations in key Notch and chromatin-remodeling genes	AL101	3mg/kg PO 4on/3off
HDAC		Vorinostat (SAHA[1])	60mg/kg IP QD
Bcl2	Bioinformatic analysis of upregulated pathways in ACC	Venetoclax (ABT-199[1])	50mg/kg PO QD
CDK4/6		Palbociclib (PD 0332991[2])	60mg/kg PO QD
FGFR		Erdafitinib (JNJ-42756493[2])	25mg/kg PO QD

Table 1: Compounds selected for in vivo combination studies

The doses were selected after completing a 14-day tolerability study in non tumor bearing Nude mice, comparing each drug alone to combination with AL101 for the effect on body weight and clinical. Compounds were purchased at [1]LC Labs or [2]MedChemExpress.

Result 3: Combination of AL101 with Compounds Targeting Bcl2, CDK4/6, FGFR & HDAC are Effective in Both Notch WT and Activated ACC Tumors

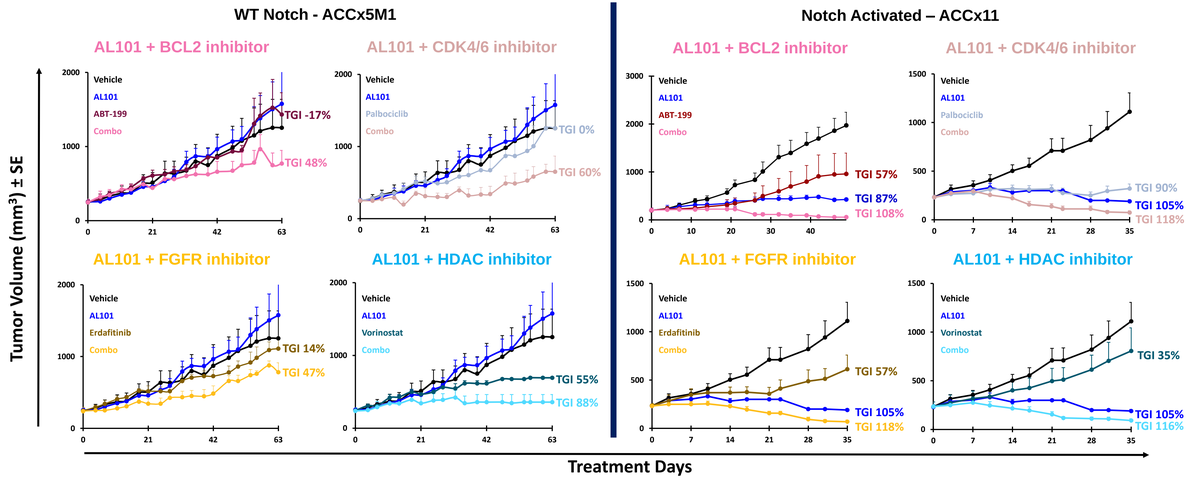


Figure 2: Effect of monotherapy versus combination therapy in ACC PDX Tumors

ACC PDX tumors (ACCRF by XenoSTART) were implanted in female athymic nude mice. Once tumors reached a size of 150-300 mm3, mice (n=3-5/group) were randomized to treatment arms (Table 1). Oral dosing with the combination compound was separated by 8 hours.

ACC = adenoid cystic carcinoma; PDX = patient-derived xenograft; SE = standard error; TGI = tumor growth inhibition; WT = wild type.

The data represents a limited number of ACC PDX models available for testing.

Conclusions

- Additive or synergistic activity of GSI combined with agents of various mechanisms of action, indicates that cross-talk between signaling pathways may increase the effectiveness of AL101 in recurrent/metastatic ACC regardless of Notch mutational status.
- This may also be a promising approach for expansion to other cancer indications in which Notch is dysregulated.

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