Plucked hair as a platform for monitoring pharmacodynamic and mechanistic consequences of clinical exposure to the Wnt/β-Catenin inhibitor PRI-724

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Introduction

The Wnt/beta-catenin signaling pathway regulates cell morphology, motility, and proliferation. Aberrant regulation of this pathway leads to neoplastic proliferation. PRI-724 is a potent, specific inhibitor of the canonical Wnt signaling pathway with potential anti-neoplastic activity. It specifically inhibits the recruitment of beta-catenin with its coactivator CBP and, together with other transcription factors, beta-catenin/CBP binds to WRE (Wnt-responsive element) and activates transcription of a wide range of target genes of Wnt/beta-catenin signaling. Blocking the interaction of CBP and beta-catenin by this agent prevents gene expression of many proteins necessary for growth, thereby potentially suppressing cancer cell growth.

Monitoring target engagement is crucial to inform on early drug development decisions, and development of a peripheral tissue-based gene expression signature of pathway inhibition could facilitate the continued clinical development of PRI-724. High vascularisation of the hair follicle, frequent epithelial origin of tumors, ease of sampling and high degree of congruence of expression in hair of pathways dysregulated in cancers, make the cellular bulb of plucked human scalp hair an excellent surrogate tissue for non-invasive monitoring of pharmacodynamic (PD) and mechanism of action (MOA) effects in clinical trials.

We have sought to identify peripheral / surrogate molecular biomarkers of target engagement and pharmacodynamic activity that can be translated to clinical use during PRI-724 development.

Methodology

Using our *ex vivo* plucked hair culture platform, hair bulbs from several healthy donors were exposed to varying doses of C-82 (active metabolite of PRI-724) over a 24 hour period. Total RNA was isolated at 6 and 24 hours post-culture from individual anagen hair bulbs and used in microarray analysis to assess global transcriptional alterations in anagen hair resulting from C-82 exposure.

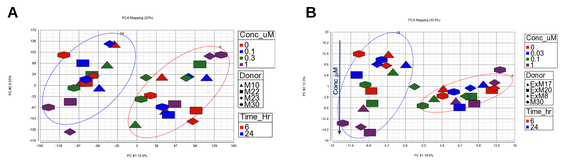

Figure 1. Initial analysis of data. A) Using all probes, the primary source of variance in the data is timepoint. B) Applying a set of probes shown to be regulated by TCF4 or β-catenin inhibition of Wnt signaling, these probes separate the 24 hour timepoint samples by increasing dose of C-82.

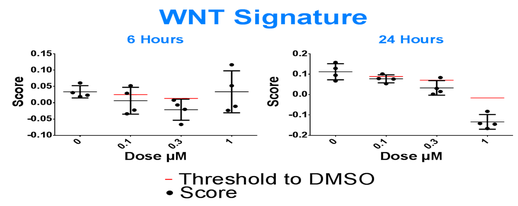

Figure 2. Wnt signature score for each hair donor transformed to mean of all samples as baseline. A multivariate Wnt signature was generated using the *a priori* defined Wnt gene set to determine the level of inhibition by C-82 of genes commonly regulated by knockdown of TCF4 or β-Catenin. Treatment with C-82 exhibited a dose-dependent decrease in signature score at the 24 hours timepoint.

A

	6 Hours			24 Hours		
	0.1 µM	0.3 µM	1 µM	0.1 µM	0.3 µM	1 µM
FDR P < 0.01	0	0	0	0	1	1898
FDR P < 0.01 & 1.5FC	0	0	0	0	1	1682
P < 0.01 & 1.5 FC	92	127	277	14	384	3874

B

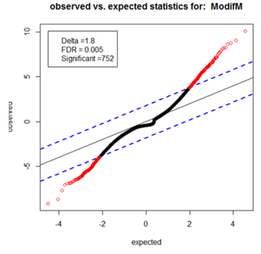

Figure 3. Summary of differential expression *ex vivo*. 3-way multivariate analysis of variance (ANOVA) was applied, using donor (random effect), time in culture and dose as experimental factors. Contrasts were performed for each pair-wise comparison of vehicle and compound treated group. A) Summary of the total numbers of probes passing combinations of statistical testing in each experimental group. A robust number of probes surviving multiple testing was only noted for the 1µM dose at the 24 hour timepoint. B) SAM analysis to identify strongest dose-responsive probes indentified a set of 589 genes FDR 0.005, with a range of differential expression from -25 fold to +17 fold at the 1 µM, 24 hour contrast, further analysis was performed using this list.

Ingenuity Canonical Pathways	-log(p-value)	Ratio	z-score	Coverage
Wnt/B-catenin Signaling	2.56	0.07	-1.41	12 of 169 Molecules

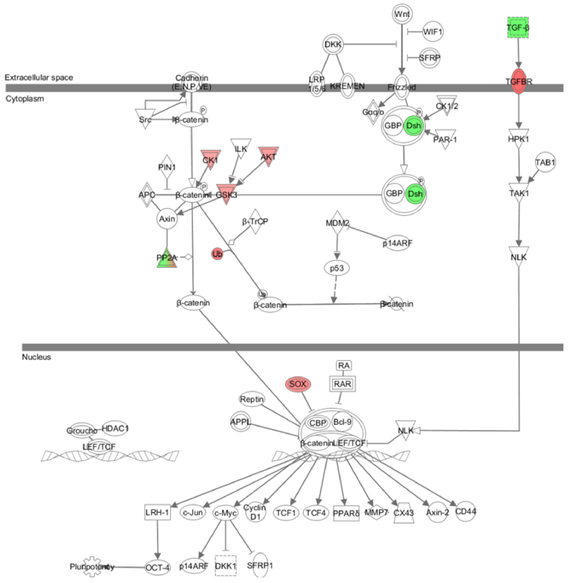

Figure 4. Engagement of WNT/β-Catenin signaling pathway in IPA. IPA predicts down-regulation of the pathway (-1.41 z-score) with 12 members of the pathway showing modulation.

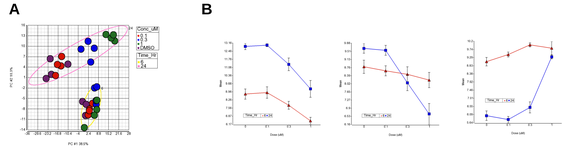

Figure 5. Dose dependent response of gene expression to treatment with C-82. A) Using the gene set as a signature separates the 24 hour timepoint treatment groups in a clearly dose-dependent manner. B) Response in expression levels to C-82 treatment in three selected genes demonstrating differences in threshold of up- and down-regulation of dose-dependent expression.

Results & Discussion

Transcriptional profiling of *ex vivo* cultured human plucked hair revealed:

- Assessment using known Wnt\β-catenin responsive genes separates *ex vivo* hair samples on a PCA plot by C-82 dose (at 24 hours) (Figure 1).
- Utilising a subset of these known probes as a putative multivariate gene signature shows strong dose dependant inhibition of “signature” by C-82 in hair (Figure 2).
- ANOVA using all probes reveals strong, statistically significant modulation of probes in a dose dependant manner (Figure 3A).
- SAM analysis to identify dose responding probes reveals strong, statistically significant (FDR 0.005) probes (589 genes, ranging from -25 to +10 fold) (Figure 3B).
- Querying IPA shows statistically significant engagement of the canonical WNT/β-Catenin signalling pathway, with 12 of 169 members modulated, with the modulation of genes showing agreement with inactivation of the pathway (Fig. 4)
- Use of the gene set as a multivariate signature separates 24 hour treatment groups in hair in a clearly dose-dependent manner (Figure 5A).
- *Ex vivo* exposure of human anagen hair to C-82 results in an identifiable and appreciable transcriptional response, which is biologically relevant to Wnt pathway modulation.

In *ex vivo* treated plucked hair, we have demonstrated an appreciable and biologically relevant transcriptional response to exposure to C-82, the active metabolite of PRI-724, reflective of the compound’s mechanism of action (MOA).

The genes identified in this study may be used to provide further MOA information in clinical settings to monitor PD responses in plucked scalp hair obtained from patients exposed to PRI-724.