#345 Predictive Software-Based Mathematical Modeling: A Novel Approach to Development of Oral Therapies for Rheumatoid Arthritis – Validation in a Murine Collagen Induced Arthritis Model.

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ABSTRACT

Background/Purpose

Rheumatoid Arthritis (RA) involves complex interactions of multiple cell systems, cytokines and mediators, and interlinked signaling. While molecularly-targeted therapies manipulate specific interactions, it is possible that other, previously redundant, paths are subsequently activated, thus causing drug resistance. Ideal therapy design requires simultaneous modulation of multiple targets to achieve eventual convergence and synergy. A predictive software algorithm would tremendously increase the ability to identify such therapies.

Methods

A predictive in-vivo equivalent simulation technology emulating RA pathophysiology at cellular and molecular level was designed by assimilating information from 8928 publications over the past 8 years. The simulation platform is a co-culture of 8 cell systems representing RA phenotypes, associated signaling and metabolic networks and includes nearly 100000 cross-talks and interactions among approximately 31366 molecules. The platform was extensively validated using more than 4000 studies and correlated with retrospective human clinical drug data and animal experiments. A digital library representing 100 targeted drugs from different indications was screened in combinations of two's and three's translating to more than one million in-vivo studies. Automated analytics engine using the assay data from the studies generated a therapy candidate which is a combination of 3 oral FDA-approved drugs (CWG952), based on criteria of efficacy, synergy and PK/PD compatibility. For in-vivo validations, mCIA was induced in male DBA/1 mice and those with paw scores between 1 and 5 on day 19 were randomly assigned to treatment arms (9/group): CWG952 and anti-TNF with treatment started at 12hrs of enrollment.

Results

The RA predictive model was dynamically simulated to disease condition followed by application of CWG952 therapy. The simulations predicted a percentage reduction in TNF, IL6, IL1B, IL17a, RANKL and CRP by 81.8, 90.5, 85.9, 86.5, 85.1 and 95.5 respectively from disease condition with respect to control state. The model also predicted a 83.1 % reduction in bone and cartilage degradation and 85.1% inhibition of osteoclast activity. Anticipated effect on phenotypic disease markers were close to those seen in clinical trials of TNF-alpha inhibitors. The figure shows results from animal experiments (mCIA model) demonstrating a comparable efficacy to etanercept.

Conclusion

In-vivo validations of mathematically-designed CWG952 show results comparable to etanercept. These results, along with other previously published validations [1], suggest a robust validation of a software-based mathematical modeling approach that incorporates the complex interaction of immunomodulating molecules, and can potentially lead to the development of innovative therapies for immunological diseases.

INTRODUCTION

- RA is a complex chronic disorder that involves cytokine deregulation that effects multiple cell-cell interactions and causes autoimmunity, chronic inflammation and joint damage.
- Current treatment of RA relies on molecularly-targeted therapies that manipulate specific interactions.
- Several patients develop resistance to these treatments over long-term, possibly because previously redundant paths are subsequently activated, thus causing drug resistance.
- Ideal therapy design requires simultaneous modulation of multiple targets to achieve eventual convergence and synergy.
- A predictive software algorithm would tremendously increase the ability to identify such therapies.
- The objective of this study is to present a predictive model to understand, represent and treat this complex disease and to design novel and multifaceted therapeutic approaches using such a system.
- A Virtual RA predictive disease model that represents the interaction of 8 key cell types involved in the pathophysiology of RA and presents RA phenotypes has been created, by mathematically connecting the insight contained in thousands of papers, to study and “treat” an intact model of the disease.
- This predictive model has been used to design a novel multi-pathway therapy, CWG952, that is designed to meet the following objectives:
  - Efficacious on multiple phenotypes
  - Efficacious in the anti-TNF refractory patient population
  - Simple and convenient oral delivery
  - Reduced potential for toxicity or side effects

METHODS AND MATERIALS

Cellworks Virtual Disease System (VDS) system: The Cellworks Virtual Disease System (VDS) system is an in-silico computational platform that simulates bio-molecular interactions, signaling and metabolic pathways representing specific cellular physiological activities.

- **Baseline Pathways and Kinetics:** VDS was created using a bottoms-up technique with extensive data mining on specific pathways in a cell (***Figure 2***). Individual interactions within different pathways are modeled via differential equations and kinetic parameters are extracted from the literature or estimated using training data.
- **Integration of Pathways**: Individual pathways are integrated with other pathways into a specific cell type. These are then trained to represent the values/trends for the function of that cell type (***Figure 1***).
- **Integration of Cell types**: Cell types are connected with each other with the key intercellular connectivity modeled. The integrated “Disease” model is validated to literature results for the specific disease condition using in-vivo/clinical data (***Figure 1***).
- **Therapy Identification**: A drug capsule library, which represents the functional activity of molecularly targeted therapies, is used to simulate the effects of individual or combinations of drugs on disease phenotypes in the Virtual RA model (***Figure 3***).

Contradictions & Missing Link Handling: Contradictions and ambiguity are two inherent aspects of biological research. During translation of pathway research data to an in silico model, several such contradictions have been encountered and resolved.

Example 1: Conflicting data on TNFa levels between Jiang D [1] and Nalbant S [2].

- For instance, Jiang D et.al showed that Celecoxib, significantly reduced the levels of TNFa[1]. However, Nalbant S et.al could show that Rofecoxib (Vioxx), increased the levels of TNFa [2]. Both are selective COX inhibitors and the results contradictory.
- Further refined searches to resolve the conflict, identified publications by Chan CC, Mastbergen SC [3],[4] (Coxibs are potent inhibitors of PGE2) and results by Xu XJ [5] (PGE2 downregulates TNFa).
- The above results gave an interpretation that both Celecoxib and Rofecoxib would increase TNFa levels via down regulation of PGE2; however the contradictory results for Celecoxib should have been obtained as a result of its unknown off-target effects.

Example 2: Data Unknown – modeling unconnected steps

- Other than contradictions, deciphering the unconnected steps in a signal transduction pathway forms an important aspect for efficient prediction of a novel consequence.
- For instance, Bettelli E et.al and Malhotra N et.al independently demonstrated that IL6 inhibits Foxp3+ Treg cell generation induced by TGFB and that IL-6 and TGFB together, induce the differentiation of pathogenic TH17 cells and that SMAD2 is essential for TGF beta mediated Th17 cell generation and IL17 secretion.
- However the exact mechanism linking SMAD2 to IL17 production was unclear [6] [7].
- A precise search for the link connecting SMAD2 to IL17 led us to the results published by Qin H et.al who demonstrated that, TGFB activated SMAD2 inhibited the inhibition of SOCS3 on STAT3, a transcription factor for IL17 and ROR-gamma, the marker for TH17 differentiation [8].
- The combination of results from the above references helped in the precise interpretation of the actual mechanism by which TGFB positively impacted IL17 production and TH17 cell differentiation.

RESULTS

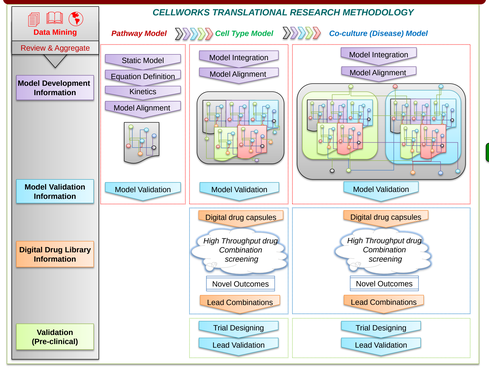
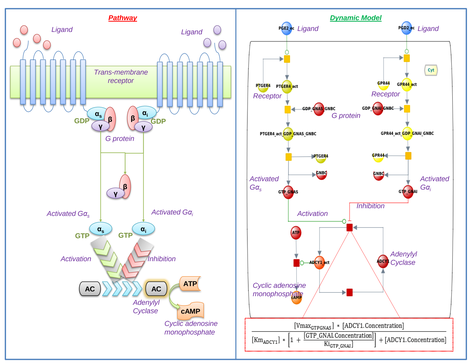


Figure 1 : Schematics depicting Cellworks translational research methodology

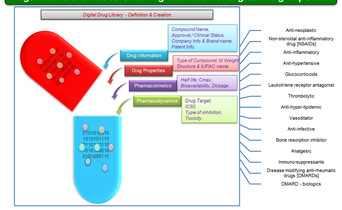
Figure 2 : Schematics showing translation of biological data to in-silico model.



Example 1 : Model Reaction Formalism for IL6 pathway

Node	Mechanism	Reaction Equation	References
Binding of IL6 ligand with IL6 receptor [TCELL_IL6R TCELL_IL6R_act] =	The reaction has been modeled as a Simple Michaelis Menten equation, with the receptor as the substrate and ligand as the activator	(((kcatf_TCELL_IL6R_act_IL6ST_di*TCELL_IL6 R_act_IL6ST_di.Concentration)*VCy)*TCELL_IL6R.Concentration)/(Km_TCELL_IL6R+TCELL_IL6R.Concentration)	Expression of IL6R on T Lymphocytes (Chen X et.al, 2010), IL6 signaling mechanism (Heinrich PC et.al, 2003), IL6 physiological role (Teague TK et.al, 1997)
Binding of IL6 ligand-receptor complex with 2 molecules of GP130 (IL6ST) [TCELL_IL6R_act + 2 TCELL_IL6ST = TCELL_IL6R_act_IL6ST_dl]	The reaction has been modeled as a Reversible Mass Action reaction with Non-Competitive inhibition from soluble GP130	((((Kf/(1+(TCELL_IL6ST_s_ec.Concentration/Ki_TCELL_IL6ST_s_ec))*TCELL_IL6R_act.Concentration)*(TCELL_IL6ST.Concentration*TCELL_IL6ST.Concentration))-(Kr*TCELL_IL6R_act_IL6ST_di.Concentration))*VCyt	Expression of GP130 on T Lymphocytes (Betz UA et.al, 1998)
Binding & Phosphorylation of JAK1 by IL6 receptor complex [TCELL_JAK1 = TCELL_IL6R_act_IL6ST_di_JAK1_p]	The reaction has been modeled as a Simple Michaelis Menten equation, with SOCS1 and SOCS3 inhibiting the phosphorylation competitively.	(((kcatf_TCELL_IL6R_act_IL6ST_di*TCELL_IL6R_act_IL6ST_di_JAK1_p*TCELL_IL6R_act_IL6ST_di_JAK1_p.Concentration)*VCy)*TCELL_JAK1_1*((1+(TCELL_SOCS1.Concentration/Ki_TCELL_SOCS1))*(1+(TCELL_SOCS3.Concentration/Ki_TCELL_SOCS3)))+TCELL_JAK1.Concentration)	SOCS inhibition of IL6 induced JAK1 phosphorylation (Fischer P et.al, 2004). Activation of JAK kinases by IL6 signaling (Narazaki M et.al, 1994)
Binding & Phosphorylation of JAK2 by IL6 receptor activated JAK1 complex [TCELL_JAK2 = TCELL_IL6R_act_IL6ST_di_JAK1_p_JAK2_p]	The reaction has been modeled as a Simple Michaelis Menten equation with IL6-JAK1p-JAK2p complex as an activator.	(((kcatf_TCELL_IL6R_act_IL6ST_di_JAK1_p*TCELL_IL6R_act_IL6ST_di_JAK1_p.Concentration)*VCy)*TCELL_JAK2_1*((1+(TCELL_SOCS1.Concentration/Ki_TCELL_SOCS1))*(1+(TCELL_SOCS3.Concentration/Ki_TCELL_SOCS3)))+TCELL_JAK2.Concentration)	Activation of JAK kinases by IL6 signaling (Narazaki M et.al, 1994)
Phosphorylation of STAT3 by the IL6 receptor activated JAK2 complex [TCELL_STAT3 = TCELL_STAT3_p]	The reaction has been modeled as a Simple Michaelis Menten equation with IL6-JAK1p-JAK2p complex as an activator.	(((kcatf_TCELL_IL6R_act_IL6ST_di_JAK1_p_JAK2_p_psu*TCELL_IL6R_act_IL6ST_di_JAK1_p_JAK2_p_psu.Concentration)*VCy)*(1+(TCELL_PRKCD_p_psu.Concentration/Ka_TCELL_PRKCD_p_psu))*TCELL_STAT3.Concentration)/(((Km_TCELL_STAT3_3)*(1+(TCELL_SOCS1.Concentration/Ki_TCELL_SOCS1))*(1+(TCELL_SOCS3.Concentration/Ki_TCELL_SOCS3)))+TCELL_STAT3.Concentration)	JAK-STAT signal transduction mechanism (Yamada S et.al, 2003), SOCS3 based negative regulation of IL6 signaling (Croker BA et.al, 2003), (Yasukawa H et.al, 2003), (Fischer P et.al, 2004), SOCS1 based negative regulation of IL6 signaling (Lee TL et.al, 2006), Role of STAT3 in T Lymphocytes (Yang XO et.al, 2007)

Figure 3 : Schematics showing definition of digital drug capsule.



Example 2 : Model Validation

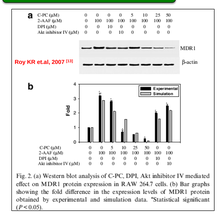
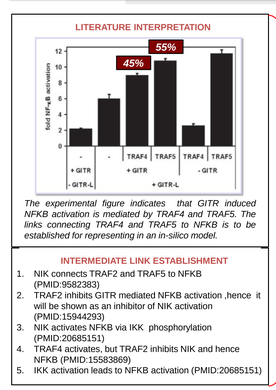


Fig. 2 (a) Western blot analysis of C-PC, DPI, Akt inhibitor IV mediated effect on MDR1 protein expression in RAW 264.7 cells. (b) Bar graphs showing the fold difference in the expression levels of MDR1 protein obtained by experimental and simulation data. *Statistical significant ($P < 0.05$).

Example 3 : Interpretation & Representation of links in in-silico model



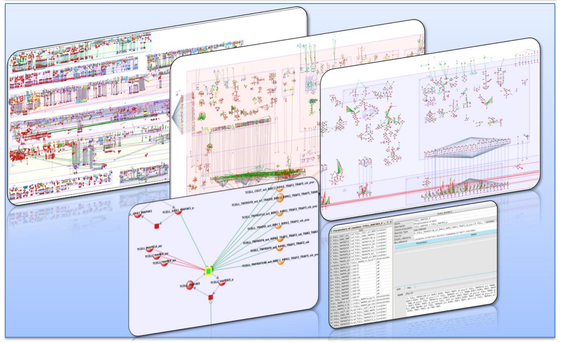
The experimental figure indicates that GITR induced NFKB activation is mediated by TRAF4 and TRAF5. The links connecting TRAF4 and TRAF5 to NFKB is to be established for representing in an in-silico model.

INTERMEDIATE LINK ESTABLISHMENT

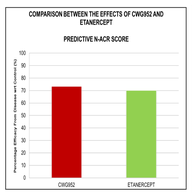
1. NIK connects TRAF2 and TRAF5 to NFKB (PMID:9582383)
2. TRAF2 inhibits GITR mediated NFKB activation ,hence it will be shown as an inhibitor of NIK activation (PMID:15944293)
3. NIK activates NFKB via IKK phosphorylation (PMID:20685151)
4. TRAF4 activates, but TRAF2 inhibits NIK and hence NFKB (PMID:15583869)
5. IKK activation leads to NFKB activation (PMID:20685151)

- ***Equation Definition***: *Since TRAF4 and TRAF5 acts as activators for the phosphorylation of NIK, the reaction has been modeled as Simple Michaelis Menten Equation.*
- ***Model Kinetics Interpolation:*** *Due to unavailability of literature support pertaining to the kinetic parameters for the activation of NIK, the turnover number (kcat) values for TRAF4 and TRAF5 have been assumed and optimized to obtain a 45% and 55% signal contributions towards NFKB as interpreted from the literature.*

Figure 4 : Hierarchical view of an in-silico multi-cell disease model.



Example 4 : CWG952 - Drug efficacy correlation between predictive and in-vivo results.



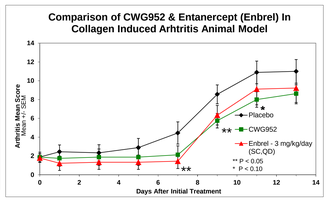


Table 2 : Predictive Efficacy data on effect of CWG952 on biomarkers.

Markers	% Reduction	Markers	% Reduction
Tumor Necrosis Factor α (TNF)	81.8	*Serum amyloid A-1 protein (SAA1)*	84.97
Interleukin-6 (IL6)	90.5	*Resistin (RETN)*	25.02
Interleukin-1 beta (IL1B)	85.9	*Adrenomedullin (ADM)*	27.35
Interleukin-17 alpha (IL17A)	86.5	*Placenta Growth Factor (PGF2)*	74.85
TNFSF11 (RANKL)	85.1	*Apolipoprotein E (APOE)*	68.84
C Reactive Protein (CRP)	95.5	*Nerve Growth Factor (NGF)*	84.85
Bone & Cartilage Destruction	83.1	*Leukemia inhibitory factor (LIF)*	68.67
Osteoclast Cell Activity	85.1	*LIGHT (TNFSF14)*	87.73
Leptin (LEP)	54.09	*TGF alpha (TGFA)*	96.17

DISCUSSION

- Ideal management of RA requires a judicious and simultaneous modulation of multiple targets/pathways to avoid resistance and be effective across multiple phenotypes with the least side effects.
- An exhaustive combinations of drugs, not feasible to test in an in-vivo setting, can be run in a cloud computing setting. Combinations are then sorted based on a complex function which takes into account the relative change across multiple disease phenotypes, impact on key biological markers etc.
- CWG952, a combination of three repurposed drugs from other indications, was identified in a screen of approximately a million combinations run over a 24 week duration and measuring efficacy impact, synergy, PK/PD compatibility. An example of the efficacy biomarker data used to identify CWG952 is shown in ***Table 2***.

CONCLUSIONS

- The Virtual Disease Model which emulates an intact disease system can facilitate in rationally designing a therapy that modulates multiple pathways to synergistically converge at multiple phenotypes and dissipate elsewhere.
- CWG952, a therapy identified by a screen in a virtual RA disease model has been validated in an in-vivo setting in a murine model (see poster 342).
- In-vivo validation of therapy: ***Example 4*** shows the correlation of the VDS predictions of efficacy relative to etanercept and the data generated from the study in murine CIA models.
- Validation of in-silico Dosage prediction: In-Silico predictions identified that the therapy would be effective against RA phenotypes when each of the individual drugs was dosed at 1/12,1/8 and 1/4th of their approved mid-level approved doses.
- In-vivo validation of dosage/synergy: CWG952 was also dosed in the murine models to achieve 10%, 16% and 50% of the plasma exposure levels of the approved dosages.

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Disclosures

C. Refino, J. Cruz: Employees of inTouch Bio who performed the in-vivo studies under contract with Cellworks Group Inc. G. Singh: Consultant, Cellworks Group, Inc. All other authors are employees of Cellworks Group Inc.

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