CAPPELLA_Combating cancer through novel approaches to protein-protein interaction inhibitor libraries

Publishable final activity report

Acronym: CAPPELLA
Project no.: LSHC-CT-2006-037251
STREP
Thematic Priority: Life Sciences

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Duration: 36 months

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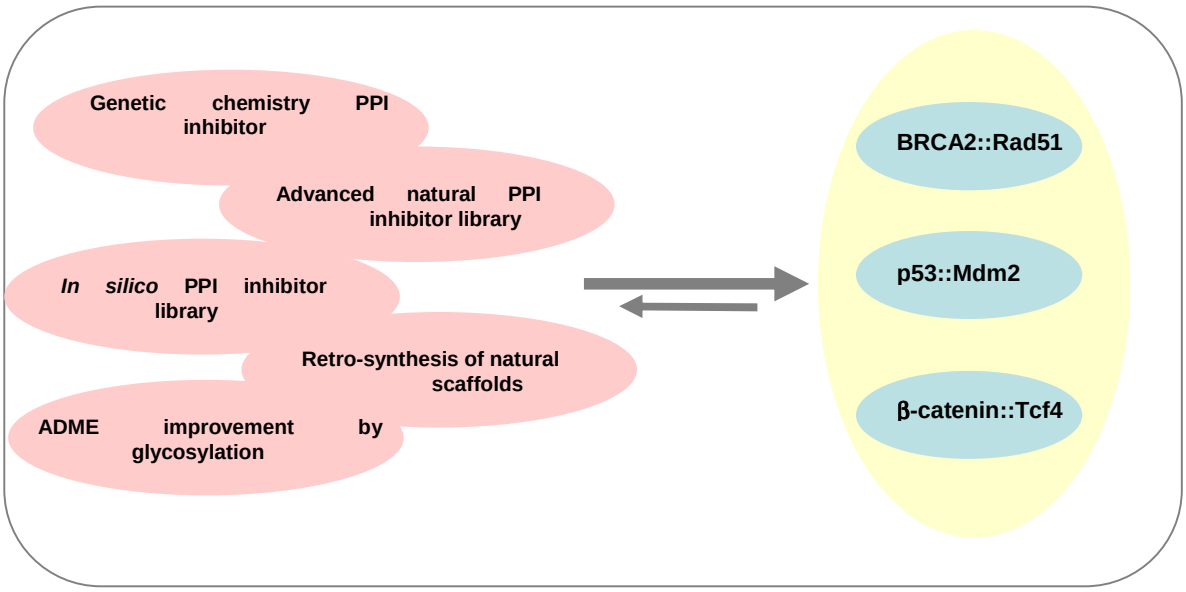
1. Project execution

Inhibition of protein-protein interaction (PPI) represents an emerging and promising approach for drug discovery and the development of novel cancer therapies. As many PPIs occur within the cell they can only be targeted by small molecule compounds. However, small molecule inhibition of PPIs represents a challenging area in drug design. As PPIs differ structurally from more classical drug targets such as enzymes and receptors, existing compounds have generally delivered disappointing results. This combined with the growing need to discover new therapeutic strategies to combat cancer calls for the development of novel and alternative approaches.

The objective of CAPPELLA is to develop a series of innovative small-ligand tools and libraries that represent new approaches to inhibit PPIs in cancer. The project is a unique opportunity to integrate novel *in-silico*, chemical, genetic and ADME-based approaches in the design, synthesis and optimization of libraries and compounds. A key theme is the utilization of structural motifs found in natural PPI inhibitor compounds. This is coupled with high content testing of the resultant structures on 3 distinct validated cancer PPI targets namely BRCA2-Rad51, p53-Mdm2 and beta-catenin-TCF4, to allow compound rule-sets to be developed and improved.

This project brings together some of Europe's leading biotech companies (AnalytiCon, IL, Evolva, PharmaMar and BioLigands) and several highly recognized academic institutions (UCPN, UCAM-DONC, UT and UNIGE). By combining 5 distinct, but complementary, chemical design approaches (see illustration below) and testing these in functional assays for the 3 different targets (all from different partners) in 3 successive but iterative cycles the project mobilizes resources from across Europe.



During the project, the Consortium has established and validated state-of-the-art high content functional assays for the 3 chosen cancer targets to facilitate screening for disruption of PPI's. These assays include a mammalian 2H assay to detect disruption of p53-Mdm2, *in vitro* assays allowing identification of beta-catenin-TCF4 inhibitors based on luciferase reporter readout and a biochemical BRCA2-Rad51 interaction assay using an ELISA format.

Screening has been conducted with > 6000 compounds derived from bio-sources of natural origin and/or marine extracts supplied by the library groups within the Consortium. For all 3 targets, primary screening and additional tests identified numerous putative compound hits, which were prioritized according to structure, activity and amenability to medicinal chemistry development. In addition, structure-based and ligand-based pharmacophore models for virtual screening of the 3 targets were built and used to screen Consortium- and commercial vendor databases providing improved compound hit list to partners.

Results from repeated cycles of screening with purchased or synthesized analogs of compound hits were fed back to compound providers for library optimization and refinement of pharmacophore models for virtual screening. Potential leads were modified by enzymatic glycosylation in an attempt to optimize their properties. At the end of the project, c. 10 interesting compound hits have been identified for the 3 targets. Iterative rounds of biological testing, pharmacophore model refinement, and virtual screening resulted in improved compound hits for at least one of the tested PPI targets (beta-catenin:Tcf4).

The biological data for the PPI inhibitory compounds was analyzed applying different computer-aided approaches and structure-activity relationships (SARs) derived from this analysis were used to define several rule-sets for PPI inhibitor compound library design. These rule-sets represent useful starting points for the development of novel drugs against the PPI targets.

The end objective of CAPPELLA, as stated in Annex I, was to identify 5-10 candidate compound families from within the tested libraries that can subsequently be taken forward into pre-clinical testing. The CAPPELLA project thereby ultimately aims to provide novel solutions for improved therapies and better treatment of various types of cancer. Compound classes that specifically disrupt PPI's could allow for the development of a range of new anti-cancer therapies against a whole series of already validated cancer targets.
As the Consortium was able to identify 6 new compound families acting on the 3 targets this end objective was successfully reached.

2. Dissemination and use

The SME's and Industry partners plan to file patent applications to new compositions and new therapeutic uses depending on the innovation (see Table below). The RTD performers will share in any value added through their contribution to the patent rights e.g. through identification of activity using their biological screens.

Dissemination of the scientific results from the project has occurred through publication in scientific journals, poster presentations and contributions at international meetings /conferences. In addition, the CAPPELLA web-site has been updated at regular intervals with scientific progress for the benefit of the general public. The final plan for using and disseminating the knowledge from the project is presented in the following 3 sections described below.

Section 1 – Exploitable knowledge and its use

Exploitable knowledge (description)	**Exploitable product(s) or measure(s)**	**Sector(s)** of application	Timetable for commercial use	Patents or other IPR protection	**Owner** & Other Partner(s) involved
Chemical structures that disrupt interaction between Rad51::Brca2	Compounds that disrupt interaction between Rad51::Brca2	Medical	2018	Patent is planned	AnalytiCon, PharmaMar, Evolva, IL, UNICAM-DONC,
Chemical structures that disrupt interaction between beta-catenin::Tcf4	Compounds that disrupt interaction between beta-catenin::Tcf4	Medical	2018	Patent is planned	AnalytiCon, PharmaMar, IL, UNIGE,
Chemical structures that disrupt interaction between p53::Mdm2	Compounds that disrupt interaction between p53::Mdm2	Medical	2018	Patent is planned	AnalytiCon, PharmaMar, IL, Bioligands

Section 2 – Dissemination of knowledge

Planned/ actual Dates	**Type**	**Type of audience**	**Countries addressed**	**Size of audience**	**Partners responsible/involved**
March 2007	*Project web-site* www.cappellabio.eu	General public	World wide		Evolva
May 2007	*Poster* Evolva technology collaborations	BIO 2007 International Convention-Boston, MA	World wide		Evolva
February 2008	*Publication* Combating Cancer through novel approaches to protein-protein interaction inhibitor libraries SME's in Health Research synopses of project funded under the call for STREPs dedicated to SME's	Higher education, SME's and wider public	EU		Evolva
March 2008	*Media briefing and Press release* Newsletters have been sent to the Danish Cancer	Research	DK, UK		Evolva

	Society and Cancer Research UK,				
March 2008	*Media briefing and Press release* CAPPELLA Newsletter has been posted on the Cancer Research UK intranet to inform their scientists of the program.	Research	UK		Evolva
April 2008	*Update of web-site* www.cappellabio.eu	General public	World wide		Evolva
June 2008	*Poster* Evolva's drug discovery programs G. Salerno, J. Hansen, T. Thybo, M. Hughes Crameri, P. Longchamp & N. Goldsmith	BIO 2008 International Convention, San Francisco	World wide		Evolva
June 2008	*Brochure* Evolva's drug discovery programs G. Salerno, J. Hansen, T. Thybo, M. Hughes Crameri, P. Longchamp & N. Goldsmith	BIO 2008 International Convention, San Francisco	World wide		Evolva
June 2008	*Publication* Combating Cancer through novel approaches to protein-protein interaction inhibitor libraries Major diseases Research Vol II	Higher education, SME's and wider public	EU		Evolva
Nov 2008	Publication S.A. Osmani, S. Bak, A. Imberty, C.Olsen, B. Lindberg Møller: Catalytic key amino acids and UDP sugar donor specificity of a plant glucuronosyl transferase, UGT94B1 *Source:* PLANT PHYSIOLOGY Volume: **148** Issue: **3** Pages: **1295-1308** Published: **NOV 2008**				

Feb 2009	*Publications* S.A. Osmani, S. Bak, B. Lindberg Møller: Substrate specificity of plant UDP-dependent glycosyltransferases as predicted by analysis of crystal structures and by homology modelling PHYTOCHEMISTRY Volume: **70** Issue: **3** Pages: **325-347** Published: **FEB 2009**	Research	World wide		UCPH
March 2009	*Update of web-site* www.cappellabio.eu	General public	World wide		Evolva
March 2009	*Publication* Substrate specificities of family 1 UGTs gained by domain swapping. Hansen EH, Osmani SA, Kristensen C, Møller BL, Hansen J. Phytochemistry. 2009 Mar;70(4):473-82.	Research	World wide		UCPH
March 2009	*Publication* S.A. Osmani, E.H. Hansen, C. Malien-Aubert, C.E. Olsen, S. Bak, B. Lindberg Møller: Effect of Glucuronosylation on Anthocyanin Color Stability. Journal of Agricultural and Food Chemistry, 2009, 57, 3149-3155.	Research	World wide		UCPH
July 2009	EC promotional poster on SME related success stories on EU funded projects in Health Presented at EuroBio	Research	Europe		Evolva
July 2009	EC promotional poster on SME related success stories on EU funded projects in Health Presented at Biotechnica	Research	Europe		Evolva
Sept 2009	*Update of web-site* www.cappellabio.eu	General public	World wide		Evolva

Sept 2009	Participation in EC coordinated Workshop on Trends in Translational Cancer Research in Europe, Bergen, Norway	Research	Europe		Evolva

Section 3 – Publishable results

Results generated during CAPPELLA on UGTs and molecular modeling, by an academic partner (UCPH), have been published in high quality peer reviewed journals (see below). This work provides significant insight into the structure-function relationship of this important group of enzymes.

Title: Catalytic Key Amino Acids and UDP-Sugar Donor Specificity of a Plant Glucuronosyltransferase, UGT94B1: Molecular Modeling Substantiated by Site-Specific Mutagenesis and Biochemical Analyses.
Author(s): Osmani SA, Bak S, Imberty A, Olsen C, Moller BL.
Source: PLANT PHYSIOLOGY Volume: 148, Issue: 3, Pages: 1295-1308, Published**:** Nov 2008

Title: Substrate specificity of plant UDP-dependent glycosyltransferases predicted from crystal structures and homology modeling.
Author(s): Osmani SA, Bak S, and Moller BL.
Source: PHYTOCHEMISTRY Volume: 70, Issue: 3, Pages: 325-347, Published: Feb 2009

Title: Substrate specificities of family 1 UGTs gained by domain swapping.
Author(s): Hansen EH, Osmani SA, Kristensen C, Moller BL and Hansen J.
Source: PHYTOCHEMISTRY Volume: 70, Issue: 4, Pages: 473-482, Published: Mar 2009

Title: Effect on glucuronosylation on anthocyanin color stability.
Author(s): Osmani SA, Hansen EH, Malien-Aubert C, Olsen CE, Bak S, and Moller BL.
Source: J. Agric. Food Chem. Volume: 57, Issue: 8, Pages: 3149-3155, Published: Mar 2009

For more information, see CAPPELLA project website: www.cappellabio.eu or contact Coordinator: Drs. Tanja Thybo/Sanne Jensen, Evolva SA.