Rational molecular assessment and innovative drug selection (RAIDs): Pharmacological profiling of 20 cervical cancer cell lines

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BACKGROUND

Recent retrospective data[1,2,3] identified major molecular alterations in cervical cancer (CC), but so far there has been no prospective assessment on patient outcome using a complete molecular profiling with quality control evaluation of treatment. The Cetuxicol (phase 2) clinical trial showed that the addition of Cetuximab over a 6 week period, did not improve DFS. PI3K pathway mutations in the tumor in the Cetuximab treatment arm led to a worse DFS[4]. We are lacking prognostic and predictive biomarkers for CC treatment and there is a growing need for the development of biomarkers to follow up the course of the disease. We performed pharmacological profiling of a panel of 20 original cervical cancer cell lines and attempt to correlate drug responsiveness with genomic markers.

METHODS

RAIDs is a multidisciplinary co-operation between academic clinical centers, SMEs and translational research platforms in seven European countries. It includes: 1) cognitive cohort study (BioRAIDs)[5], intended to define patient stratification for targeted therapies; 2) a targeted clinical trial using an HPV directed vaccine and 3) preclinical studies aiming at assessing new treatment strategies

Reverse phase protein array to assess signaling pathways involved in CC was performed on more than a 150 patient and 20 CC cell lines at Institut Curie (France).

Bioinformatics pipelines to detect somatic mutations and clustering methods were developed in order to stratify the patients into different subgroups.

Systematic screening of drugs on miniaturized cell-based assay was performed on 20 original cervical cancer cell lines. 43 different drugs have been tested, singly or in combination with Paclitaxel and Carboplatin for their ability to induce cell death.

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RESULTS

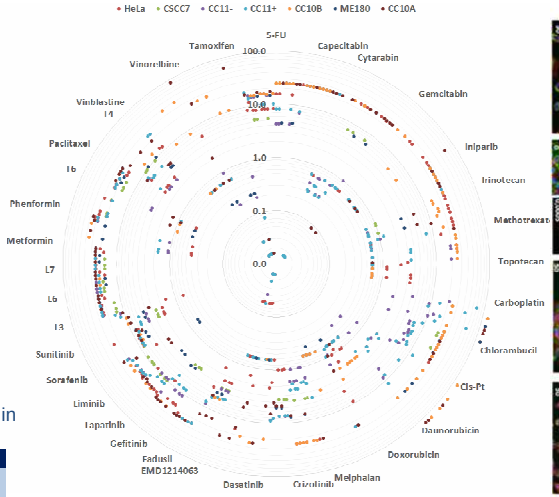
Table1. Number of significantly mutated genes by cell line (TSG= Tumor suppressor gene

CC Cell line	Number of mutated genes	Oncogenes	TSG
CRL10302	798	2	11
CRL1550	530	1	6
CRL1594	592	1	11
CRL1595	558	1	7
CRL2614	638	1	4
CRL7920	580	4	6
HTB31	2096	7	14
HTB32	528	1	6
HTB33	501	2	6
HTB34	498	3	3
HTB35	594	1	3
IC1	556	1	3
IC3	446	3	7
IC4	562	1	4
IC5	594	1	5
P26	911	3	9
P49	929	2	11
P55	802	3	9
P71	728	1	3
P82	694	1	4

Table 2. Driver mutated genes and activated pathways in 4 CC cell lines (Ingenuity pathways analysis)

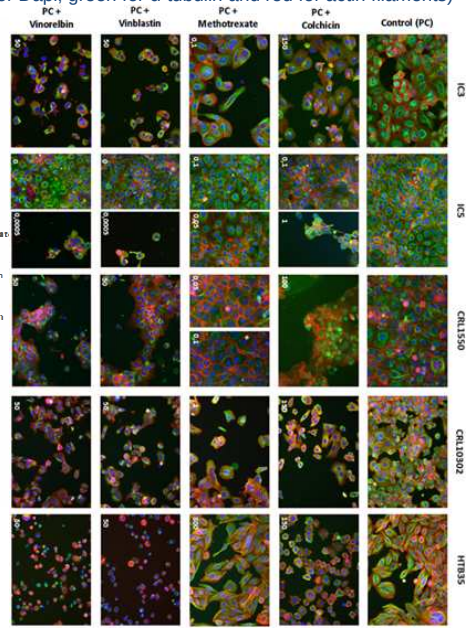
	Gene	Classification	Core_pathway
IC1	FGFR3	Oncogene	PI3K; RAS ; STAT
	ATM	TSG	DNA Damage Control
	MLL3	TSG	Chromatin Modification
	NOTCH2	TSG	NOTCH
	ERBB2	Oncogene	PI3K; RAS
	GATA2	Oncogene	NOTCH, TGF-b
	SMO	Oncogene	Hedgehog
IC3	ARID1B	TSG	Chromatin Modification
	MAP3K1	TSG	RAS; MAPK
	MLL3	TSG	Chromatin Modification
	NOTCH2	TSG	NOTCH
	PBRM1	TSG	Chromatin Modification
	RNF43	TSG	APC
	TNFAIP3	TSG	Cell Cycle/Apoptosis; MAPK
IC4	ERBB2	Oncogene	PI3K; RAS
	MLL3	TSG	Chromatin Modification
	PTCH1	TSG	Hedgehog
	SETD2	TSG	Chromatin Modification
	STK11	TSG	mTOR
IC5	ERBB2	Oncogene	PI3K; RAS
	ATM	TSG	DNA Damage Control
	BRCA2	TSG	DNA Damage Control
	CIC	TSG	RAS
	MAP3K1	TSG	RAS; MAPK
	MLL3	TSG	Chromatin Modification

Figure 1. Drugs tested on the 20 CC cell lines generating ~20000 data points & 800 IC50 determined - Dynamic range 10nM to 40mM



The profiling consists in determining IC50 of 43 drugs either alone or in combination with paclitaxel/carboplatin which is the standard of care treatment for recurrent or metastatic disease.

Figure 2. The morphology of CC cells treated with three-drug combinations for 48h (20X). Staining (blue for Dapi, green for α-tubulin and red for actin filaments)



CONCLUSIONS

- A group of drugs that synergizes with the "standard treatment" has now been identified and these molecules interfere with different signaling circuitries including epigenetic mechanisms (HDAC), the functioning of receptor tyrosine kinases (EGFR, VEGFR/PDGFR) and nuclear hormone receptors (tamoxifen) as well as the energy metabolism (biguanides).
- The bioinformatics analysis to link the drug responsiveness of cervical cancer cell lines to genomic alterations identified through the exome sequencing is currently being performed.
- These data will constitute a pharmacogenomics resource that will be used to develop pre-clinical models and to propose therapeutic hypotheses in order to match the treatment according to the genetic profile of the tumors