Abstract # 3093

Sensitive Detection of Gamma-H2AX Induction As A Pharmacodynamic Marker For Profiling Cancer Patients Treated With Topotecan

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Abstract

Background: Chemotherapies for cancer patients are often designed to inhibit growth or induce apoptosis in malignant cells. Several genotoxic agents have proved effective in inducing significant DNA damage, such that apoptosis is triggered in the cell. Topotecan is a chemical inhibitor of DNA topoisomerases. The inhibition of topoisomerases blocks the ligation of the phosphate backbone of DNA and leads to the generation of strand breaks. In this study we evaluated gamma-H2AX as marker for drug efficacy, using antibodies that recognize the phosphorylated S139 epitope.

Results: We presented an automated blood-based assay for detecting phosphorylated gamma-H2AX before and after drug treatment. We first screened subpopulations of the blood for cell types that exhibit the greatest response in gamma-H2AX induction after drug treatment. Using this sub-phenotype, we then developed two approaches for isolating these cell types: immunomagnetic enrichment of the top responding cell types, and immunophenotyping of the top responding cell subtypes. We measured gamma-H2AX, using laser scanning cytometry (LSC) and found that the induction magnitude of the biomarker (1) correlates to treatment with the topotecan in set of *ex vivo* patient samples and in clinical trial patients; and (2) is at least as sensitive as manually measurements of gamma-H2AX foci using conventional methods in comparison of 10 patient samples. We have begun a clinical validation study to determine whether this induction correlates to clinical outcome and additionally are investigating whether early response to topotecan, in the form of biomarker induction, is predictive of outcome. Preliminary results in 10 patient samples show that the early gamma-H2AX response to DNA damage is highly variable from patient to patient, ranging from no measurable induction, to an induction of 65%.

Conclusions: We have developed an automated system for measuring gamma-H2AX induction in cancer patients treated with topotecan. Studies correlating the marker to outcome are ongoing. If this assay is predictive, it would be invaluable for rapidly stratifying patients in clinical trials that involve therapies that induce DNA damage.

Background

- **Phosphorylated H2AX (γH2AX) has been reported as a biomarker for DNA double-strand breaks and programmed cell death**
- **Increase in expression of γH2AX in circulating tumor cells in topotecan-treated patients has been reported[1]**
- **Release of soluble γH2AX in *ex vivo* topotecan-treated PBMC has also been reported[2]**
- **However, sensitivity of different PBMC sub-populations to the induction of γH2AX has not been determined**
- **Laser scanning cytometry (LSC) is a versatile, robust, quantitative cell imaging system that provides high content cytometric information on single cells as well as on populations**
- **We hypothesize that (1) different PBMC sub-populations may be selectively sensitive to γH2AX induction, and (2) PBMCs collected from patients treated with topotecan may have elevated level of γH2AX expression**

γH2AX is Increased in Topotecan-Treated PBMC Subpopulations

- **PBMCs isolated from healthy individuals were treated with topotecan**
- **Increased γH2AX expression was detected in different lymphocyte populations. The lymphocyte populations with the greatest induction in γH2AX expression were:**
  - **CD14+ (max 117%)**
  - **CD3+ (max 145%)**
  - **CD16b+ (max 313%)**

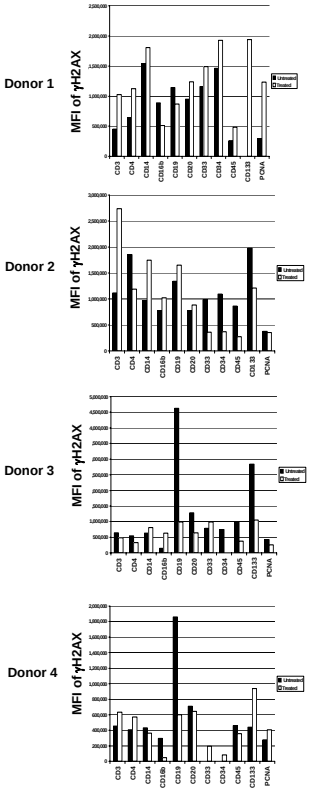

Figure 1. Expression of γH2AX in lymphocyte subsets in topotecan-treated or untreated PBMCs.

Cross-Validation of the γH2AX Assay – Apocell and NCI

➢Objective:

•To test the variation of results using the same procedure performed at two different laboratories

➢Procedure:

•Cell line (SR and THP1) samples were treated with or without cyclophosphamide at NCI and sent to Apocell
•Samples were then stained and scanned at Apocell, and then scanned at NCI
•The same samples were also stained at NCI and scanned at NCI, and then scanned at Apocell

➢Results:

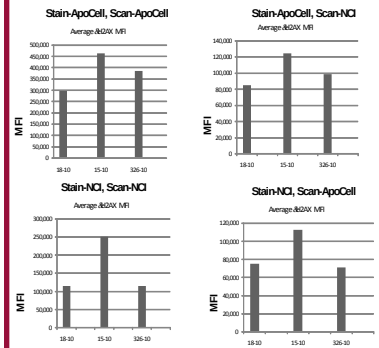

Figure 2 . Cross-laboratory validation of the assay. Same pattern in γH2AX expression level was observed by operators at NCI and at Apocell

References

1.Wang L.H., Pfister T.D. et al. Monitoring drug induced γH2AX as a pharmacodynamic biomarker in individual circulating tumor cells. Clinical Cancer Research 16:1074, 2010

2.Zhang Y. Parchment R.E. et al. Quantitative γH2AX immunoassay for pharmacodynamic monitoring of DNA damage by chemotherapeutic agents or radiation. AACR abstract #1763, 2010

Increase in γH2AX Level in CD3/CD14+ PBMC Isolated from Some Topotecan-Treated Patients

➢Objective:

•To test if γH2AX expression levels in PBMCs harvested from topotecan-treated patients

➢Procedure:

•Whole blood samples were collected from cancer patients before and after topotecan treatment

•PBMCs were isolated, fixed and shipped to Apocell for analysis

➢Results:

Patient ID	Time Points	Number of DAPI positive cells	Number of CD3/CD14/γH2AX positive cells	% of CD3/CD14/γH2AX positive cells	γH2AX MFI from CD3/CD14/γH2AX positive cells	γH2AX Index	% change compared with Pre dose
1	Day 1, 0 Hr	396	353	89.2	251,876	24975345	
	Day 1, 4 Hr post	434	420	96.8	202,851	21830742	-21
	Day 1, 0 Hr	1059	1003	94.7	226,296	21402945	-14
	Day 3, 4 Hr post	532	592	109.0	288,670	29867000	15
	Day 3, 0 Hr	283	278	98.2	275,892	27102758	9
	Day 5, 4 Hr post	1659	1645	99.2	232,402	23057979	-8
2	Day 1, 0 Hr	446	125	28.0	76,864	2154260	
	Day 1, 4 Hr post	1453	573	40.8	90,264	3687294	71
	Day 3, 0 Hr	2166	641	29.6	81,164	2401945	11
	Day 3, 4 Hr post	NA	NA	NA	NA	NA	NA
	Day 5, 0 Hr	689	676	98.7	222,883	21554922	901
	Day 5, 4 Hr post	5456	300	7.1	46,767	334438	-84
3	Day 1, predose	239	238	99.6	566,327	54366743	
	Day 1, postdose	239	179	74.9	503,973	37745256	-33
	Day 4, postdose	239	113	47.3	367,681	17384981	-68
4	Day 1, predose	58	46	79.3	316,966	25138683	
	Day 1, postdose	35	49	70.0	252,420	17684453	-30
5	Day0, predose	962	799	83.1	139,883	11918143	
	Day 1, postdose 2 hrs	424	408	96.2	260,039	25022525	115
	Day 1, postdose 4 hrs	729	598	82.1	142,016	11655053	0
	Day 1, postdose 6 hrs	769	659	85.7	142,353	12199041	5
	Day 1, postdose 24 hrs	NA	NA	NA	NA	NA	NA
	Day 2, predose	968	698	73.6	144,409	10623922	-9
6	Day 1, predose	488	402	82.9	183,998	14808873	
	Day 1, postdose	457	341	90.8	290,853	24309374	64

Table 1. Measurement of γH2AX in CD3+/CD14+ cells at pre-dose and post-treatment time points in patients treated with topotecan. Three out of six patients had elevated γH2AX expression after dosing.

Conclusions

- **We have successfully developed an LSC-based assay to measure γH2AX within CD3+/CD14+ lymphocyte populations.**
- **We are also currently evaluating examination of γH2AX from immunomagnetic isolated CD3+/CD14+ cells**
- **This assay has the potential to be developed as a pharmacodynamic assay to monitor drug activity in cancer patients undergoing chemotherapy.**

γH2AX Detection in Immunomagnetic Bead-Enriched CD3+ and CD14+ Cells

➢ **Objective:**
- **To determine if immunomagnetic bead isolation procedure could be used to isolate lymphocyte subsets from blood collected into CellSave tubes and if γH2AX expression could be measured from the isolated cells.**

➢ **Procedure:**
- **PBMCs, from healthy donors, were isolated from blood collected into CellSave tubes**
- **CD3+ cells, CD14+ cells, or CD16b+ cells were enriched by immunomagnetic bead separation**
- **Enriched cells were stained for γH2AX and levels of expression were measured by LSC**

➢ **Results:**

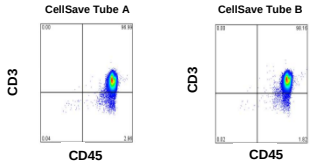

Figure 3. Immunomagnetic bead capture of specific sub-populations of PBMCs in blood samples collected into CellSave tubes to enrich lymphocyte subsets. Shown here is the flow cytometry profile of enriched CD3+ T cells from 2 individuals (A and B).

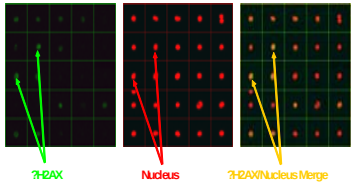

Figure 4. Images of γH2AX staining, DAPI (nucleus) and merged images of both

Sample ID	Total Cell # (Nucleus)	?H2AX Positive Cells	?H2AX MFI in Total Cell	?H2AX MFI in ?H2AX Positive Cells
CellSave R&D	636	163	405,036	1,424,503

Table 2. Measurement of γH2AX in CD3+/CD14+ enriched PBMCs

➢ **γH2AX expression can be detected in immunomagnetic bead enriched CD3+/CD14+cells**