ECRONICON OPEN ACCESS

MICROBIOLOGY
Research Article

In Vitro Generated Tumor/APC Hybrids Induce Allogeneic Tumor-Killer T Cells

Yehia S Mohamed[1,2]*, Kamel A El Ghareeb[2], Fatma A Gomaa[2] and Eman Y Abu-rish[3]

[1]*Department of Microbiology, College of Medicine, University of Dammam, Saudi Arabia*

[2]*Department of Microbiology and Immunology, Faculty of Pharmacy, Al-Azhar University, Egypt*

[3]*Department of Biopharmaceutics and Clinical Pharmacy, Faculty of Pharmacy, University of Jordan, Jordan*

***Corresponding Author:** Dr. Yehia S Mohamed, Department of Microbiology, College of Medicine, University of Dammam, Dammam, Saudi Arabia.

Received: October 13, 2015; **Published:** October 21, 2015

Abstract

This study evaluates the ability of human hybrid cells, generated by fusion of multiple myeloma (MM) primary tumor cells and immortalized antigen presenting cells (APC), HMy2 (EBV sensitized B-lymphoblastoid cell line), to present tumor antigens in context of MHC class I and class II, and to maintain expression of the co-stimulatory molecules to prime tumor-specific T cells with the ability to recognize and destroy parent MM cells *in vitro*. Bone marrow-derived MM cells were fused to HMy2, *in vitro*, and the growth of the hybrid cells was chemically selected and maintained under the tissue culture conditions. Hybrid cells cloning to a single cell via plate serial dilution was carried out before the phenotypic evaluation using flow cytometer. Tumor antigen expression by the hybrid cells was detected through RNA extraction followed by reverse transcription PCR using antigen specific primers. Subsequently, hybrid cells were allowed to induce T cell specific response from PBMCs of normal healthy donors. Cellular cytotoxicity and IFN-γ ELISpot assays were performed to evaluate the specificity and functional activity of the activated T cells. The hybrid cells showed remarkable ability to express relevant tumor antigens in context of MHC I/II, in addition to T cell co-stimulatory molecules. The generated hybrid cells also induced allogeneic cellular T cell responses of both cytotoxic $CD8^+$ and $CD4^+$ subtypes. More importantly, the induced cytotoxic $CD8^+$ T cells successfully released IFN-γ and recognized the target tumor cells in ELISpot and cellular cytotoxicity assays. We concluded that hybrid cells of this type had the potential to induce specific T cell response and could be a successful immunotherapeutic, T cytotoxic cells inducer to be used for adoptive cellular management of hematologic cancers.

Keywords: *Multiple myeloma immunotherapy; Adoptive T cell transfer; Tumor-specific T cells; Hybrid cell vaccines*

Abbreviations: APC: Antigen presenting cell; BSA: Bovine serum albumin; CD: Cluster of differentiation; cDNA: Complementary deoxyribonucleic acid; CTL: Cytotoxic T lymphocytes; DCs: Dendritic cells; ELISpot: Enzyme linked immunospot; EBV: Epstein Barr virus; FACS: Fluorescence- activated cell sorter; FITC: Fluorescein isothiocyanate; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; HAT: Hypoxanthine-aminopterin –thymidine; HLA: Human leukocyte antigen; IFN-γ: Interferon γ; IF: Immunofluorescence; LCL: Lymphoblastoid cell line; MHC: Major histocompatibility complex; mAb: Monoclonal antibody; MLR: Mixed lymphocyte reaction; MM: Multiple myeloma; mRNA: Messenger RNA; NK: Natural killer; PBLs: Peripheral blood lymphocytes; PBMC: Peripheral blood mononuclear cells; PE: Phycoerythrin; RNA: Ribonucleic acid; RPMI 1640: Roswell Park Memorial Institute medium 1640; RT–PCR: Reverse transcription – polymerase chain reaction; rhIL-2: Recombinant human interleukin-2; TAAs: Tumor associated antigens; TC: Tissue culture

Introduction

Hematological malignancies represent around 10% of all malignancy cases and denote high mortality rates worldwide. Due to recent advances in controlling and management of the disease, a better reduction in annual mortality rate was achieved in the last few years [1]. In the recent years, a substantial interest has been provided for developing new immunotherapeutic approaches for cancer management. Much of these efforts have focused on the ability of antigen presenting cells (APCs) for activating tumor-specific T-cell responses [2]. APC/tumor cell hybrids have been shown to induce protective immunity to certain induced tumors in animal models and, in some cases,

to eradicate established tumor masses [3-6]. However, fusion of dendritic cells (DCs; the most powerful APC) and tumor cells results in hybrid cells that are short-lived, with limited replicative ability, have variable fusion efficiencies, and also have incomplete characterization profiles.

Moreover, adoptive T-cell transfer (ACT) represents an alternative approach to active immunization, as a mean of cancer immunotherapy. This immunotherapeutic approach has been used with significant clinical benefits in EBV-associated lymphoid disorders [7,8], myeloma [9], and leukemia [10,11], in addition to a group of solid tumors. These and other studies [12,13] have demonstrated the probability of generating CTL lines to high manufacturing standards, as well as the tolerability and therapeutic benefit of the approach.

In previous studies it has been shown that the EBV-associated B lymphoblastoid cell line (LCL), HMy2 [14], was fused to human tumor cells and cell lines *in vitro* to produce stable hybridoma cells that grew spontaneously in tissue culture, expressed tumor-associated antigens (TAAs), and showed an enhanced ability to stimulate allogeneic T cell responses [15]. Moreover, these studies prospered in the induction of tumor antigen-specific cytotoxic T-lymphocytes (CTL) in peripheral blood lymphocytes (PBL) from healthy donors, and semi-autologous cancer patients as detected by HLA-A2-peptide pentamer staining and cellular cytotoxicity assays [16,17]. Hybrid cell lines, such as those described, could be used as *in vitro* stimulators of antigen-specific CTL for adoptive cellular transfer. In this study, we generated hybrid cell by fusion of multiple myeloma primary tumor cells with HMy2 cell line; the fusion product showed phenotypic profile similar to parent APC, regarding expression of co-stimulatory markers and HLAs, and simultaneously expressed tumor antigens of the parent tumor cells. These hybrids survived immortally in tissue culture and when co-cultured with allogeneic normal PBMC, triggered primary T cell responses with tumor-specific cytotoxic potency as revealed by IFN-ϒ release ELISpot and ^{51}Cr-release cellular cytotoxicity assays. Our data indicate that this model of hybrid cell lines could be a candidate for use in tumor immunotherapy, by in vitro generation of tumor-specific T cell clones for adoptive cell transfer.

Materials and Methods

Human blood or bone marrow sample cells were collected using signed consent forms, and after getting the local Institutional Review Board approval (IRB2013-05-018; King Fahd Hospital of Dammam University) and were used in accordance to the ethical standards of the appropriate institutional and national committees and with the Helsinki Declaration of 1975 (revised in 2008).

Tissue culture (TC)

The in vitro growth of multiple myeloma tumor cells (MM), APC and the generated hybrid cell lines (Table 1) were maintained in tissue culture conditions using the recommended culture media, RPMI 1640 supplemented with 100 IU/ml penicillin, 100 µg/ml streptomycin and 2 mM L-glutamine (all from Sigma Aldrich, UK), and in standard tissue culture condition, using 5% CO_2 in humidified atmosphere at 37°C. Primary cells were store in liquid nitrogen until further use.

Cell line name	Description
MMxHMy2	HMy2 hybrid of multiple myeloma
HMy2	EBV- B lymphoblastoid cell line
K562	Chronic myeloid leukemia cell line

Table 1: *APC (HMy2), hybrid and NK target (K562) cell lines that were used in the study.*

Generation of the hybrid cells

Tumor cells of MM were fused to EBV-lymphoblastoid cell line; HMy2 [14] using PEG/ DMSO 1500 w/v as described before [15]. Following fusion, cell mixture was transferred to chemical selection growth medium, which contains hypoxanthine, aminopterin and thymidine (HAT), for 3 days to select the growth of heterohybrid cells. Both homohybrids and non-fused cells are unable to grow in presence of these selective agents, in addition; MM tumor cells couldn't survive in TC conditions for more than a week.

Evaluation of the fusion efficiency

Fusion efficiency was evaluated before and after the chemical selection through immunofluorescence (IF) staining and flow cytometry of the fusion product using monoclonal antibodies (mAb) for lineage-specific markers, CD_{138} and CD_{19} for MM and B-lymphoblastoid lineages respectively, where the true hybrid show double positive fraction.

Cloning of the generated hybrid cells

The true hybrid cells (expressing both lineage-specific markers) were cloned to single cells by means of plate serial dilution method using ultra low attachment 96-well plates (Corning, USA), as mentioned in the manufacturer's protocol.

Phenotypic characterization of the generated hybrid cells

The phenotypic properties of 2 independent clones of the fusion product were evaluated for HLAs and co-stimulatory markers expression using direct IF staining with the relevant mouse anti-human mAbs (Table 2), where fluorescently labeled isotype antibodies were used as negative control, followed by acquisition of the stained-cells by the flow cytometer (FACSCalibur, BD, UK) and data were analyzed using CellQuestPro software (BD, UK). Moreover, hybrid cells were assessed for expression of two selected tumor associated antigens (TAA), MAGE-A3 and NY-ESO-1, through total RNA extraction using RNeasy mini kit (Qiagen, UK), reverse transcription of the extracted RNA into complementary first strand cDNA using single strand transcription kit (Invitrogen, UK), followed by antigen-specific reverse transcription polymerase chain reaction (RT-PCR) using primer sequences and cycling settings as previously described for MAGE-A3 and NY-ESO-1 [17], where GAPDH was used as a housekeeping gene. Following RT-PCR, the amplification products were separated by gel electrophoresis and visualized by UV transillumination (UVP, USA).

mAb	Isotype	Labell.	Final conc.	Source
Isotype control	IgG1	FITC/PC5	0.5 µg/ml	BD, UK
Isotype control	IgG1	PE	0.5 µg/ml	BD, UK
HLA-ABC	IgG1	FITC	0.5 µg/ml	BD, UK
HLA-A2	IgG1	FITC	0.5 µg/ml	BD, UK
HLA- DR, DP, DQ	IgG1	FITC	0.5 µg/ml	BD, UK
CD80	IgG1	FITC	0.5 µg/ml	BD, UK
CD86	IgG1	PE	0.5 µg/ml	BD, UK
CD40	IgG1	FITC	0.5 µg/ml	BD, UK
W6/32	IgG1	plain	0.5 µg/ml	BD Biosciences, UK
L-243	IgG1	plain	0.5 µg/ml	BD Biosciences, UK
Isotype control	IgG1	plain	0.5 µg/ml	BD Biosciences, UK
CD138	IgG1	PE	0.5 µg/ml	BD, UK
CD19	IgG1	FITC	0.5 µg/ml	BD, UK

Table 2: *Monoclonal antibodies that were used in the immunofluorescence staining and lymphoproliferative roles of MHC class I and II of HMy2, parent and hybrid cells.*

Lymphopropliferative abilities of the hybrid cells

Co-culturing of selected, Mitomycin-C treated, hybrid cells or relevant parent tumor cells with allogeneic PBMCs (obtained by Lymphoprep separation of heparinized peripheral blood of healthy volunteer donors) was carried out in a 6-days culture in ration 3:1 responder to stimulator cells, to evaluate the lymphoproliferative and naïve T cell priming capabilities of these hybrid clones. At the fifth day of the experiment, radioactive thymidine was added 1 µl/well, plates were incubated overnight and then harvested and counted for the radioactive content as mentioned before [17]. HMy2 cells stimulated and un-touched PBMC were used as positive and negative control wells respectively.

Induction of cytotoxic T cell response

The cloned hybrid cells were additionally investigated for priming of specific T cell response by co-culturing the Mitomycin-C treated hybrids with allogeneic PBMC (obtained as mentioned in last section) in ratio 3:1 responder to stimulator cells for 4 weekly rounds in 6-well TC plate at concentration of 3 x 10^6 responder cell/ml in supplemented RPMI-1640 growth media, where fresh stimulator cells and media were added every 7 days in presence of rhIL-2 (BD, UK) in a concentration of 300 IU/ml starting from 2nd week. By the end of fourth week, the activated T cell cultures were harvested, counted and subjected to evaluation of tumor specificity and functional cytotoxic activity.

Functional evaluation of the activated T cells

The proliferating CD_8 or CD_4 T cells induced after co-culturing with the hybrid cells were subjected to specificity and functional-activity evaluation using IFN-γ release ELISpot and tumor-specific ^{51}Cr release cytotoxicity assays.

ELISpot assay

ELISpot assay was performed using human IFN-γ ELISpot kits (Mabtech AB, Sweden), according to the manufacturer's instructions. Briefly, wells were coated with capture antibody (anti-IFN-γ clone 1-D1K) and incubated overnight at 4°C, cell were washed and counted and responder cells were added in triplicate wells, and Mitomycin-C-treated stimulator cells were added in a ratio of 3:1 responder to stimulator cells in a total volume of 200 µl/well. Plates were incubated at 37°C in 5% CO_2 for 48h. After incubation, biotinylated anti-IFN-γ mAb (clone7-B6-1) was added to the washed wells followed by two hours incubation; finally, Streptavidin-conjugated alkaline phosphatase was added for one hour. Spots were developed by addition of BCIP/NBT substrate, and were counted under an inverted light microscope using a x40 objective lens. The data were presented as mean number of spots per 10^6 responder cells ± SEM, based on triplicate wells per culture condition.

Chromium (^{51}Cr) release cytotoxicity assays

Cytotoxicity assays were carried out using ^{51}Cr-labeled MM tumor cells, HMy2, or MMxHMy2 hybrid cells as targets. Target cells were labeled with 100 µCi ^{51}Cr (PerkinElmer, UK), washed and seeded in 96-well round bottom plates in triplicate wells. Effector cells, from long-term T cell cultures were added in the desired Effector: target cell ratios to a final volume of 200 µl/ well of supplemented growth media, and plates were incubated for 4h at 37ºC in a humid 5% CO_2 incubator, before harvesting and counting the radioactivity of the supernatant. Specific lysis was calculated by applying the following formula:

$$\frac{Experimental\ Release\ -\ Spontaneous\ Release}{Maximum\ Release\ -\ Spontaneous\ Release}\times 100$$

Statistical analysis

Statistical analysis was carried out using paired t tests or ANOVA as appropriate, using PRISM 4 software (GraphPad, San Diego, CA, USA). A value of $p < 0.05$ was taken as signifying a statistically significant difference.

Results

Fusion efficiency and stability of the generated hybrid cells

Multiple myeloma tumor cells isolated from patient's bone marrow aspirate sample were fused with EBV-sensitized B-lymphoblastoid cell line, HMy2 [14]. The chemical selection (using HAT supplemented RPMI-media) of the generated true hybrid cells (double positive for lineage markers) gave rise to fusion efficiencies of 67% and 98% representing percentage of true hybrids before and after the chemical selection process respectively as revealed by the flow cytometric analysis (dot plots not shown). The selected hybrid cells grew continuously in tissue culture in single cells or suspended small clumps (similar to the lymphoblastoid partner; HMy2) which required passage of 1:4 every 3 days, and characterized by homogenous morphology (Figure 1), which survived subsequent freezing-thawing cycles.

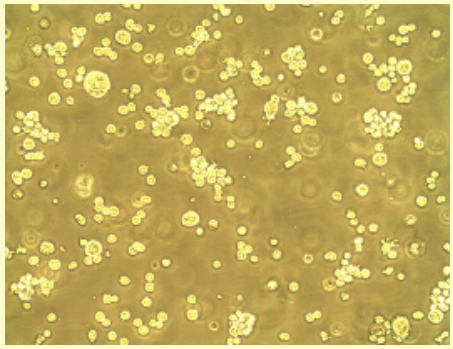

Figure 1: *Morphological Features of the Generated Hybrid Cells. Morphologic features of MMxHMy2 cells generated by in vitro fusion of MM tumor cells with HMy2 cells and cultured in RPMI-1640 growth medium. Light inverted microscopic photo (x 200).*

CD markers and HLA phenotyping of selected hybrid clones

The expression of CD markers and HLAs by parent tumor cells, HMy2 cell line and 2 clones of the generated hybrid were assessed using direct IF staining with the relevant mAbs, and analyzed by flow cytometer.

HMy2 cells expressed high levels of CD40, CD80 and CD86, MHC class I and class II, and HLA-A2, which the normal is known surface phenotype [15]. The hybrid cell clones showed consistent high expression level of CD40, CD80, CD86, HLA class I, HLA-A2 and HLA class II, which was similar, but not equal, to HMy2 expression level. Whereas, parent AML tumor cells showed low expression level of CD80, CD86 and HLA class II, but no expression at all of CD40, (Figure 2). This data of differential phenotype for the generated hybrids, parental fusion partners (HMy2 and MM tumor cells), imply (to a great extent) a true hybridization product.

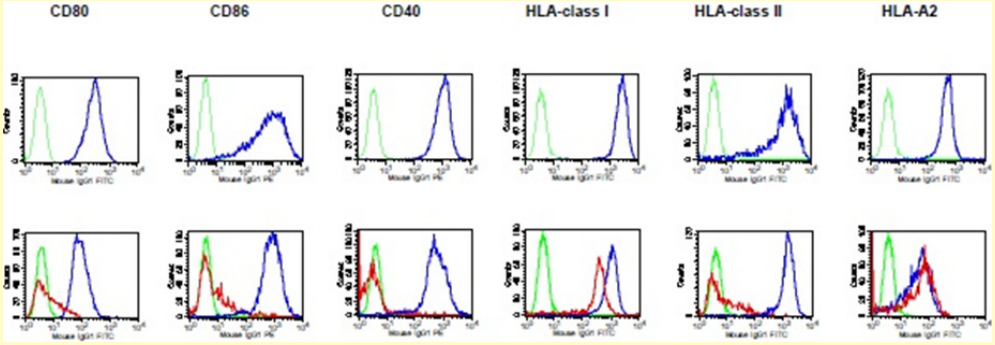

Figure 2: *CD Markers and HLA Expression Profile. The expression profile of surface HLA class I, HLA-A2, HLA class II, CD40, CD80, and CD86 by HMy2 (top panel), hybrid (blue histograms) and parent tumor cells (red histograms) (bottom panel) respectively. Data were obtained by flow cytometric analysis of relevant mAb-stained cells, and analysis was carried out using CellQuestPro software following gating on living cell population.*

Tumor associated antigen expression

TAA expression was investigated using antigen-specific RT-PCR for MAGE-A3 and NY-ESO-1 antigens, where both antigens are cancer testis antigens and highly expressed by hematologic malignancies with no revealed expression in normal tissues except by immune privileged germ line tissues; GAPDH was used as housekeeping gene. HMy2, hybrid cells and parent tumor cells were evaluated, and

all the three cell lines expressed NY-ESO-1 antigen at high level; on the other hand, the hybrid cells expressed MAGE-A3 in noticeably higher level than both HMy2 and tumor cells (Figure 3). Depending on differential CD marker and TAA expression profile of the generated hybrid cells, we expected a high allogeneic (and/or tumor-specific) immunostimulatory capability of the generated hybrid cells; so, we started to induce T cell cultures using the phenotyped hybrid cells.

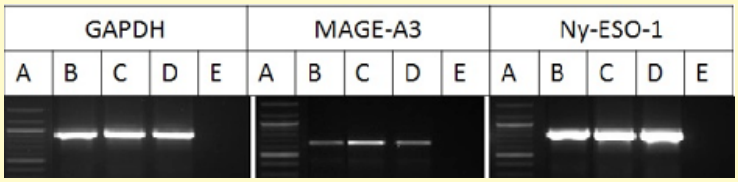

Figure 3: *RT-PCR Bands for TAA Expression. RT-PCR bands for TAA (MAGE-A3 and NY-ESO-1) expression by HMy2 (B), hybrid cells (C) and MM tumor cells (D). GAPDH was used as a house keeping gene, the PCR runs were normalized using cDNA-free well (E) as negative control. ; All positive bands were at the predicted size for the antigen-specific PCR, 100 bp lane is 50 base pair ladder (A).*

Assessment of lymphoproliferative capability of the hybrid cells

Mixed lymphocyte reaction (MLR)

T-cell cultures were established by *in vitro* stimulation of allogeneic PBMCs using tumor cells, HMy2 cells, or the generated hybrid cell lines as stimulator cells, un-stimulated PBMCs were used as control wells for result normalization. Both of HMy2 and hybrid cells showed a robust abilities to induce allogeneic (or tumor specific) lymphoproliferative responses, which were highly significant than the response to parent tumor cells alone. No significant difference was seen between response to MM tumor-stimulated and un-stimulated PBMC, Figure 4A.

Role of MHC class I and class II presentation

To see the role of MHC molecules' antigen presentation on T cell proliferation, we repeated the MLR under the same conditions except for addition of IgG1 isotype mAb, W6/32 (anti-human MHC class I), L-243 (anti-human MHC class II) or both of W6/32 and L-243 to the stimulator cells before mixing with the responder PBMC. As seen in Figure 4B, addition of IgG1 isotype had no change on the proliferative abilities of HMy2 or hybrid cells; however, W6/32 addition reduced the PBMC proliferation to a significant level which was more significant in case of HMy2 than hybrid cells; on the other hand, L-243 addition didn't affect the level of PBMC proliferation in response to hybrid cells significantly, but had a little effect of HMy2 proliferative level. Finally, addition of both of W6/32 and L-243 together reduced the level of proliferation dramatically in both of HMy2 and hybrid cells, but no significant reduction of PBMC responses to parent tumor stimulation which was weak even before mAb addition.

Induction and functional assessment of long-term induced T cell culture

HMy2, the hybrid and its parent tumor cell partners were used separately, under optimized *in vitro* tissue culture setting, to stimulate PBMC from healthy donors. After 4 weekly-rounds of co-culture, the induced T-cells from HMy2 or MMxHMy2 cultures were assessed for the presence of allo- or MM-specific cytotoxic T lymphocytes using IFN-γ release ELISpot and ^{51}chromium release cytotoxicity assays. Whereas, T cells from MM culture couldn't survive more than a week.

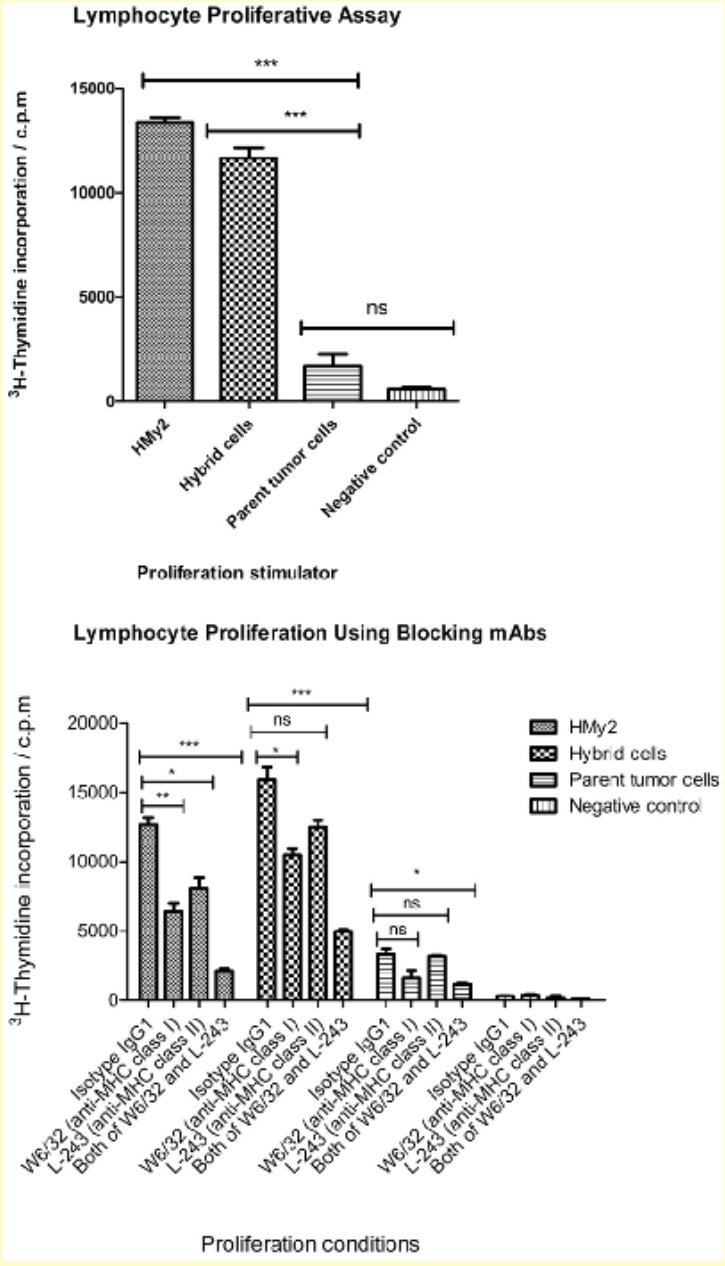

Figure 4A & 4B: *Lymphoproliferative Assay with or without blocking monoclonal antibodies. Lymphoproliferative responses as a result of in vitro stimulation of allogeneic PBMC, from healthy donors, with different stimulator cell types. HMy2, MMxHMy2 or parent tumor cells were used independently as stimulator cells in 96-well plate cultures. Proliferation level was measures by counting radioactive thymidine incorporation of the harvested T cells. 4A: represents response without blocking mAb; however, 4B: represents independent experiment of proliferative responses in presence of: Isotype IgG, W6/32 (mAb for human MHC class I), L-243 (mAb for MHC class II), or both of the mAbs. Error bars represent standard error of mean of triplicate cultures. Statistically significant differences are illustrated by asterisks (ns where $p \geq 0.05$; ** $p < 0.01$, *** $p < 0.001$). All experiments were carried out using responder T cells from (at least) 2 healthy individuals, with consistent results. HMy2 cells were included as a positive control for allogeneic T-cell stimulation, and un-stimulated PBMC as negative controls.*

IFN-γ release ELISpot Assays

Using naïve PBMC or hybrid cultures-activated T cells as responders and HMy2, hybrid or parent tumor cells as stimulator cells, ELISpot test was carried out as 48 hours incubation period, using un-touched T cells as background control wells. As in Figure 5, data show a significant increase in IFN-γ release in case of hybrid culture-activated T cells than in naïve PBMC; however, the huge difference in response of activated compared to naïve T cells was significant ($p < 0.001$) in case of HMy2 and hybrid stimulator cells, and was less significant ($p < 0.01$) in case of MM tumor stimulator cells. The increase in functional and cytokine-releasing T cells might be attributed to induction of specific T cells to the allogeneic or tumor antigens expressed by the hybrid cells. Background control, however, showed minimal responses in both cultures.

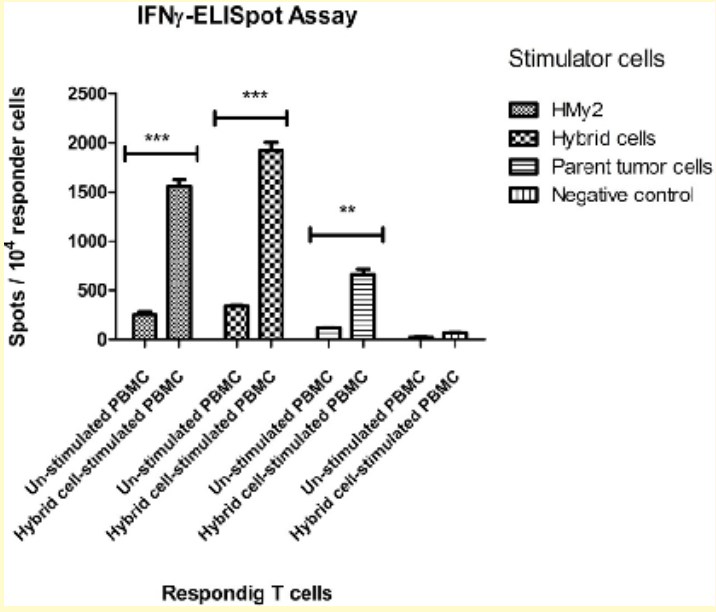

Figure 5: *IFN-γ Release ELISpot Assay. IFN-γ ELIs pot assay using allogeneic non-primed or hybrid-primed T cell cultures as responders, and HMy2, hybrid cells, or parent tumor cells as stimulators in 48h plates. The emerged spots were presented as spot number per 1 x 10^4 responder cells. Data are presented as mean of triplicate wells ± SEM. Stars show the degree of significance of difference between responses to primed and non-primed T cell cultures, where *p < 0.05; **p < 0.01; ***p < 0.001.*

Chromium (^{51}Cr) release cytotoxicity assay

In this assay, T cells from hybrid cell-cultures were investigated for their functional cytotoxic capability as effector cells. In this experiment, cytolytic activity was investigated against the relevant MM tumor cells, as well as the stimulatory hybrid cell line or HMy2 cells, in addition to K562 cell line (as NK target cells) independently, Figure 6. The CTL response against ^{51}Cr-labelled target cells was measured at 3 ascending effector: target ratios of 3:1, 10:1, and 30:1. The results show that activated T cells were able to recognize and eradicate the MM cells, in addition to HMy2 and hybrid cells; however, little specific cytotoxic activity was seen against NK target, K562 cells, Figure 6. This may indicate the involving of a tumor-specific CTL clone (or clones) in the hybrid-activated T cell culture, which might be also associated with other allo-specific clones.

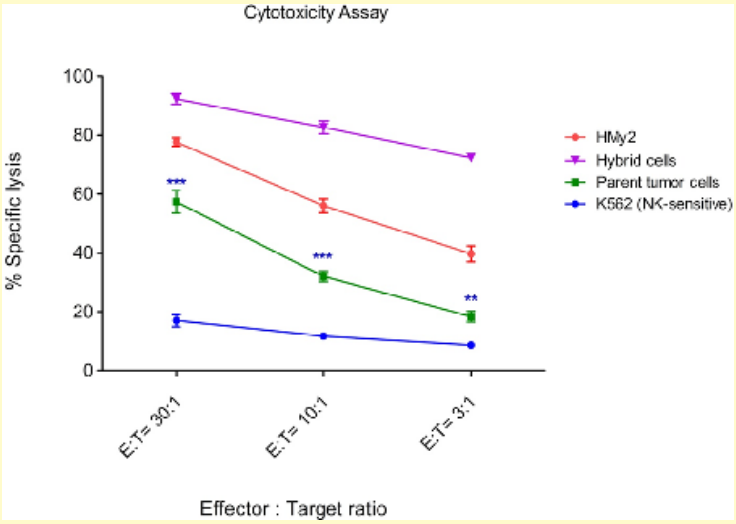

Figure 6: *Cellular Cytotoxicity Assay. Tumor-specific cytotoxicity test using allogeneic MMxHMy2-primed T cell culture as responder cells. The target MM parent tumor, HMy2, MMxHMy2 hybrid, and K562 cells were labeled with ^{51}Cr and plated at three different effector/ target ratios (3:1, 10:1, and 30:1) in 4 hour-cytotoxicity assay. K562 cell line was used as NK target to show the NK activity within the effector cell culture. Data represent mean ± SEM of specific cell lysis of triplicate wells. Stars show the degree of significance of difference between specific lysis of parent MM tumors and NK-target (K562) cells (Analysis was implemented using two way ANOVA; where *p < 0.05; **p < 0.01; ***p < 0.001).*

We couldn't obtain sufficient multiple myeloma- patient's blood to evaluate the autologous PBMC responses to stimulation with the generated hybrid cells.

Discussion

Tumor cell-APC hybrid vaccines are regarded as a good immunotherapeutic candidate for tumor management. They are getting more interest due to their ability to activate host immune cells against unique tumor antigens expressed in context of MHC class I and II, in addition to expression of T cell co-stimulatory molecules [2], and especially when combined with other agents targeting immunosuppressive checkpoints. The fact that the hybrid cells activate cytotoxic T cells in PBMC from healthy individuals confirms that the hybrid cells directly triggered naive T cell responses, a feature limited to professional APCs. In a discrete paper, we have shown that in vitro stimulation of PBMC from 2 hematological malignancy patients, from whose tumor cells hybrid cell lines had been generated, with semi-autologous LCL/tumor hybrid cells induced T cell lines with cytotoxic activity against the patients' own tumor cells [16], admiring the ability of LCL/tumor hybrid cell lines to induce tumor-specific CTL responses in hematologic malignancy patients. Moreover, in vivo animal models and human trials showed the induction of anti-tumor specific immune responses [19], as well as getting clinical progress by using APC/tumor hybrid model [20]. One of the recent papers showed the induction of cytotoxic myeloma-specific immune responses using autologous APC/melanoma cell hybrid *in vivo* [21], and a similar finding was published for renal cell carcinoma patients [22]. In addition, semi-autologous APC/tumor model has also proved to induce immune-specific responses in animal models [23], as well as in human trials [24].

In summary, this study shows the possibility of generating long-lived, self-replicating hybrid cell lines by fusion of the B-lymphoblastoid cell line, HMy2, with multiple myeloma tumor cells. The generated hybrid cell lines expressed relevant tumor associated antigens (MAGE-A3 and NY-ESO-1) in context of MHC class I and class II, and T cell co-stimulatory markers, which effectively stimulated primary allogeneic T-cell responses of both CD_8 and CD_4 subtypes as indicated by the drop in proliferative response with addition of

MHC blocking monoclonal antibodies. And by long-term co-culture, hybrid cells induced specific cytotoxic T-cells in PBMC from healthy individuals *in vitro*, these T cells exhibited both cytokine release and effective cellular cytotoxic potency against relevant tumor cells as well as other relevant cells (HMy2 and hybrid cells), which imply the possibility of tumor as well as allogeneic specificity. The ability of hybrid cells to survive immortally and to present parent-relevant TAA antigens in a way which enhances induction of specific T cells, might introduce them as candidate for tumor immunotherapy through the induction of allogeneic or even syngeneic (which share some MHC alleles with the hybrid cells) tumor reactive cytotoxic T cells on large-scale and be used therapeutically for adoptive cellular transfer in semi-autologous or allogeneic setting. Consequently these hybrid models represent candidate agents for use in immunotherapy of hematological malignancies, alone or in association with other immunomodulators.

Conclusion

From these data, it can be concluded that the tumor cell fusion to HMy2 cells enhanced the tumor cells' immunogenicity and ability to induce primary functional and specific cellular T cell responses. Although the antigen-specificity of the activated cytotoxic T cells was not confirmed, there might be certain myeloma tumor-specific clones, in addition to other clones specific for major and minor histocompatibility antigens expressed by the stimulator hybrid and HMy2 cell lines. Moreover, in addition to allogeneic- responses, some TAA-(which were investigated in the study by the hybrid cell line, or other antigens) -specific CTLs might have been generated, and in order to confirm this, further investigation shall be done on the level of antigen specific T cells.

Bibliography

1. Siegel R., *et al*. "Cancer statistics 2013". *CA Cancer Journal for Clinician* 63.1 (2013): 11-30.
2. Browning MJ. "Antigen presenting cell/ tumor cell fusion vaccines for cancer immunotherapy". *Human Vaccine Immunotherapy* 9.7 (2013): 1545-1548.
3. Gong J., *et al*. "Induction of antitumor activity by immunization with fusions of dendritic and carcinoma cells". *Nature Medicine* 3.5 (1997): 558-561.
4. Wang J., *et al*. "Eliciting T cell immunity against poorly immunogenic tumors by immunization with dendritic cell-tumor fusion vaccines". *Journal of Immunology* 161.10 (1998): 5516-5524.
5. Siders WM., *et al*. "Induction of specific antitumor immunity in the mouse with the electrofusion product of tumor cells and dendritic cells". *Molecular Therapy* 7.4 (2003): 498-505.
6. Yasuda T., *et al*. "Superior anti-tumor protection and therapeutic efficacy of vaccination with allogeneic and semiallogeneic dendritic cell/tumor cell fusion hybrids for murine colon adenocarcinoma". *Cancer Immunology Immunotherapy* 56.7 (2007): 1025-1036.
7. Haque T., *et al*. Allogeneic cytotoxic T-cell therapy for EBV-positive post transplantation lymphoproliferative disease: results of a phase 2 multicenter clinical trial. *Blood* 110.4 (2007): 1123-1131.
8. Heslop HE., *et al*. "Long-term outcome of EBV-specific T-cell infusions to prevent or treat EBV-related lymphoproliferative disease in transplant recipients". *Blood* 115.5 (2010): 925-935.
9. Rapoport AP., *et al*. "Restoration of immunity in lymphopenic individuals with cancer by vaccination and adoptive T-cell transfer". *Nature Medicine* 11.11 (2005):1230-1237.
10. Marijt E., *et al*. "Phase I/II feasibility study evaluating the generation of leukemia-reactive cytotoxic T lymphocyte lines for treatment of patients with relapsed leukemia after allogeneic stem cell transplantation". *Haematologica* 92.1 (2007):72-80.
11. Warren EH., *et al*. "Therapy of relapsed leukemia after allogeneic hematopoietic cell transplantation with T cells specific for minor histocompatibility antigens". *Blood* 115.19 (2010): 3869-3878.
12. Turin I., *et al*. "GMP production of anti-tumor cytotoxic T-cell lines for adoptive T-cell therapy in patients with solid neoplasia". *Cytotherapy* 9.5 (2007): 499-507.
13. Meehan KR., *et al*. "Development of a clinical model for ex vivo expansion of multiple populations of effector cells for adoptive cellular therapy". *Cytotherapy* 10.1 (2008): 30-37.

14. Edwards PA., *et al*. "A human-hybridoma system based on a fast-growing mutant of the ARH-77 plasma cell leukemia-derived line". *European Journal of Immunology* 12.8 (1982): 641-648.
15. Cywinski AL., *et al*. "Hybrid cells formed by fusion of Epstein-Barr virus-associated B-lymphoblastoid cells and either marrow-derived or solid tumour-derived cell lines display different co-stimulatory phenotypes and abilities to activate allogeneic T-cell responses *in vitro*". *Tissue antigens* 68.2 (2006): 115-126.
16. Mohamed YS., *et al*. "*In vitro* evaluation of human hybrid cell lines generated by fusion of B-Iymphoblastoid cells and *ex vivo* tumour cells as candidate vaccines for haematological malignancies". *Vaccine* 30.46 (2012a): 6578-6587.
17. Mohamed YS., *et al*. "Long-lived fusions of human haematological tumour cells and B-lymphoblastoid cells induce tumour antigen-specific cytotoxic T-cell responses in vitro". *Immunobiology* 217.7 (2012b): 719-729.
18. Katz T., *et al*. "Dendritic cell cancer vaccines: from the bench to the bedside". *Rambam Maimonides Medical Journal* 5.4 (2014): e0024.
19. Li Z., *et al*. "Cancer immunotherapy: are we there yet?" *Experimental Hematology and Oncology* 2.1 (2013): 33.
20. Tada Y., *et al*. "Analysis of cytotoxic T lymphocytes from a patient with hepatocellular carcinoma who showed a clinical response to vaccination with a glypican3derived peptide". *International Journal of Oncology* 43.4 (2013): 1019-1026.
21. Rosenblatt J., *et al*. "Vaccination with dendritic cell/tumor fusion cells results in cellular and humoral antitumor immune responses in patients with multiple myeloma". *Blood* 117.2 (2011): 393-402.
22. Avigan DE., *et al*. "Phase I/II study of vaccination with electrofused allogeneic dendritic cells/autologous tumor-derived cells in patients with stage IV renal cell carcinoma". *Journal of Immunotherapy* 30.7 (2007): 749-761.
23. Wells JW., *et al*. "Semi-allogeneic dendritic cells can induce antigen-specific T-cell activation, which is not enhanced by concurrent alloreactivity". *Cancer Immunology Immunotherapy* 56.12 (2007): 1861-1873.
24. Marten A., *et al*. "Allogeneic dendritic cells fused with tumor cells: preclinical results and outcome of a clinical phase I/II trial in patients with metastatic renal cell carcinoma". *Human Gene Therapy* 14.5 (2003): 483-494.

Volume 2 Issue 3 October 2015
© All rights are reserved by Yehia S Mohamed., *et al*.