Serum Apolipoprotein E and Other Inflammatory Markers Can Identify Non-Responding Patients to a Dendritic Cell Vaccine1,2

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Abstract

BACKGROUND: Despite the majority of patients do not gain any benefit from dendritic cells (DC) vaccines, this approach has occasionally given rise to dramatic responses in melanoma. Biomarkers are crucial to identify which patients are more likely to respond. We looked for correlations between pre- or post- vaccination biomarkers and clinical outcomes to DC therapy in a cohort of patients with stage IV melanoma receiving a vaccine with autologous ex-vivo expanded DCs pulsed with allogeneic tumor cell lysate. METHODS: Serial serum samples were collected at baseline, week 4 and 12 and they were analyzed for a panel of different inflammatory markers using cytometric bead array technology and ELISA. RESULTS: Twenty-one patients were evaluable for response. Patients were separated into responders and non-responders based on clinical benefit. Responders were defined as patients who achieved a complete response, partial response or stable disease the latter lasting for at least 6 months. Responders (N = 9) showed a significantly longer Progression-free Survival (PFS; HR 0.23; 95% CI 0.08–0.62; P < .001) and Overall Survival (OS; HR 0.22; 95% CI 0.08–0.59; P < .001). The clinical non-responder phenotype correlated with an elevated pre-vaccination level of cytokines associated with inflammation compared to clinical responders (Apolipoprotein C111; IL-12 p40; MiP1α; Stem Cell Factor and TNFα). Apolipoprotein E (ApoE) was also significantly elevated in the pre-vaccine sera of the clinically non-responding group and in addition it was found to correlate with outcomes. Patients with increased levels of ApoE had a significantly shorter PFS (HR 3.02; 95% CI 1.09–8.35; P = .015) and OS (HR 2.40; 95% CI 0.9–6.3; P = .034). CONCLUSION: Our findings support the notion that treating the inflammatory background may have an impact on clinical outcome for patients receiving immunotherapy. A larger study is needed to confirm the significance of ApoE as a predictive biomarker for response to DC vaccines.

Translational Oncology (2019) 12, 397–403

Introduction

Autologous dendritic cell (DC) vaccines have been used in the past 25 years for the treatment of cancer with mixed outcomes [1]. Although good clinical responses have been reported and a DC-based vaccine has even been granted approval by the FDA for the use in advanced prostate cancer [2], the vast majority of the studies resulted in objective response rates of less than 10% with the clinical benefit generally limited to a period of about 3 months.

A number of different reasons have been suggested to explain why DC vaccines do not produce better clinical results. Among these reasons are (1) the complexity of the isolation and differentiation/
maturation procedures involved in vaccine preparation (2) the variability of the antigen loading and DC maturation protocols (3) the source and type of antigen used and (4) the presence of an inflammatory immunosuppressive tumor microenvironment that cannot be overcome by the DC-based vaccine.

In this respect melanoma patients with elevated inflammatory markers such as raised LDH, lymphocyte/neutrophil ratio, C-reactive protein [3] and inflammatory cytokines such as IL-6 and IL-1ra have a generally poor prognosis and tend not to respond to treatment [4,5].

A few years ago we completed an early phase study with autologous ex-vivo expanded DCs pulsed with allogeneic tumor cell lysate in patients with metastatic melanoma to assess the safety and the feasibility of this approach. We collected serial serum samples from the patients on study to evaluate panel inflammatory markers and we report the results here.

Materials and Methods

Clinical Trial

This was a phase I/II study of autologous ex-vivo expanded DCs pulsed with allogeneic tumor cell lysate in patients with unresectable metastatic melanoma. The study was approved by the Local Ethics Committee at St George’s Hospital Medical School (Ethics Committee reference number: 03.0285) and the endpoints were primarily safety and feasibility. Patients with stage IV melanoma (either treatment naïve or pre-treated) with an ECOG ps 0 and feasibility. Patients with stage IV melanoma to assess the safety and the feasibility of this approach. We collected serial serum samples from the patients on study to evaluate panel inflammatory markers and we report the results here.

Samples were collected up to 72 hours prior to the first vaccine and at week 4 and 12 on study prior to administration of the vaccine. Ten milliliters of blood was collected into EDTA tubes by venepuncture. Tubes were centrifuged at 2000G for 10 minutes and serum was subsequently aspirated from the cell pellet. Serum was frozen at -70°C within 1 minute of separation and stored until use.

Dendritic Cells Isolation and Differentiation

One unit of peripheral blood was taken from study participants by apheresis, centrifuged to isolate the cell pellet and labeled with Clinimax CD14 beads according to the manufacturer’s instructions (Miltenyi). Cells were isolated using the Enrichment 1.1 protocol on the Clinimax apparatus. Isolated CD14 cells were washed in phosphate buffered saline (PBS) and then differentiated using IL-4 and GM-CSF (Peprotech and Leukomax respectively). CD14+ cells were cultured in 25 ml of RPMI1640 supplemented with 5% human AB serum (Gibco) in T175 flasks at 1-2 × 106 cells ml-1 with GM-CSF (50ngml-1) and IL-4 (10ngml-1) for 7 days. Cytokines were recovered from storage in liquid nitrogen by thawing in a 37°C water bath over 100 s, before being re-cultured for 24 h.

Serum Collection

Samples were collected up to 72 hours prior to the first vaccine and at week 4 and 12 on study prior to administration of the vaccine. Ten milliliters of blood was collected into EDTA tubes by venepuncture. Tubes were centrifuged at 2000G for 10 minutes and serum was subsequently aspirated from the cell pellet. Serum was frozen at -70°C within 1 minute of separation and stored until use.

Tumor Lysate Preparation and DC Loading

Generation of monocyte-derived DCs, pulsing with tumor cell line derived lysates and freeze/thaw maturation process have been detailed elsewhere [6]. Melanoma tumor lines (KM, MJT3 and NF) were derived lysates and freeze/thaw maturation process have been detailed elsewhere [6]. Melanoma cells were irradiated with 150Gy and then lysed by repeated freeze/thaw cycles. Lysates were assessed for residual cell viability using Trypan Blue staining and were passed through 0.2 μm filters to remove cell debris. Protein concentration was determined by Bio-Rad protein assay kit. Tumor

Table 2. Patients’ Characteristics

| Age/ Gender | Stage AJCC v.7 | ECOG PS | BOR Treatment-naive | PFS (months) | OS (months) |
|-------------|----------------|---------|---------------------|--------------|-------------|
| 64 male     | Ib             | 0       | SD                  | No           | 7           | 18 months   |
| 50 male     | Iv              | 0       | CR                  | Yes          | 85 months - Alive | 85 months - Alive |
| 75 male     | Ia             | 0       | PR                  | Yes          | 24          | 80 months – Alive |
| 38 male     | Iv              | 1       | SD                  | No           | 6           | 38 months   |
| 48 male     | Iv              | 0       | SD                  | Yes          | 6           | 18 months   |
| 60 male     | Iv              | 0       | SD                  | No           | 6           | 10 months   |
| 45 male     | Iv              | 0       | PR                  | Yes          | 16          | 75 months   |
| 50 male     | Iv              | 0       | SD                  | No           | 7           | 14 months   |
| 30 male     | Iv              | 0       | CR                  | No           | 81 months - Alive | 81 months - Alive |
| 53 male     | Iv              | 0       | SD                  | No           | 4 months    | 16 months   |
| 73 male     | Iv              | 1       | SD                  | Yes          | 4 months    | 15 months   |
| 60 male     | Iv              | 0       | PD                  | Yes          | 5 months    | 11 months   |
| 55 male     | Iv              | 0       | PD                  | No           | 3 months    | 10 months   |
| 67 male     | Iv              | 0       | PD                  | No           | 3 months    | 4 months    |
| 49 female   | Iv              | 0       | PD                  | No           | 3 months    | 10 months   |
| 49 male     | Iv              | 1       | PD                  | Yes          | 3 months    | 7 months    |
| 70 male     | Iv              | 0       | PD                  | No           | 3 months    | 7 months    |
| 74 male     | Iv              | 0       | PD                  | Yes          | 3 months    | 5 months    |
| 70 male     | Iv              | 1       | PD                  | Yes          | 3 months    | 3 months    |
| 60 male     | Iv              | 0       | PD                  | No           | 5 months    | 5 months    |
| 54 male     | Iv              | 0       | PD                  | No           | 4 months    | 4 months    |
| 63 male     | Iv              | 1       | NE                  | Yes          | NE          | NE          |

BOR: Best Overall Response, SD: Stable Disease, PR Complete Response, PD Progressive Disease; PFS: Progression-free survival; OS: Overall Survival; NE: not-evaluable.
lysate was stored at −80°C until used. On day 7 DCs were re-plated at 3x10^6 cell ml$^{-1}$, tumor lysate was added to a final concentration of 100μgml$^{-1}$ and cells cultured for 2 hours in RPMI 1640, supplemented with 5% human AB serum (Gibco) at 37°C. IL-4 and GM-CSF were added for a further 2 hours and then cells were harvested, aliquoted and stored in liquid nitrogen for subsequent use.

**Serum Biomarker Analysis**

Cytometric bead array was performed by Rules Based Medicine (RBM; see website for details: www.rulesbasedmedicine.com/) using their basic Human Multi-Analyte Profile (MAP) array. Briefly, a multiplex bead array system was employed to determine the levels of a number of markers, including cytokines and chemokines, present in the serum of patients. A complete list of analytes is shown in Table 1.

The concentration of Apolipoprotein E3 (ApoE3) in sera was measured with an in-house ELISA. Ninety-six-well NUNC maxisorp microtiter plates were coated with 50 μl/well of 1 μg/ml rat anti-human ApoE3 (MAB41441, R & D Systems, UK) in PBS overnight at 4°C. The plates were washed 4 times with PBS containing 0.05% Tween (PBS-T) and then blocked with 300 μl/well PBS containing 1% bovine serum albumin (BSA, Sigma, UK) for 1 hour at room temperature. After 4 washes with PBS-T, 50 μl/well human recombinant ApoE3 standard (4144-AE, R & D Systems, UK) diluted serially from 1000 to 0.1 ng/ml and human serum samples diluted 1/1000 (all in PBS containing 1% BSA) were added to the plates in triplicate and incubated for 2 hours at room temperature. After 4 washes with PBS-T, 50 μl/well goat anti-human Apo E (AF4144, R & D Systems, UK) diluted to 1 μg/ml was added and incubated overnight at 4°C. After 4 washes with PBS-T, 50 μl/well rabbit anti-goat IgG-HRP (R & D Systems, UK) at 1/1000 was added and incubated for 30 minutes at room temperature. The plates were washed a further 4 times with PBS-T and 2 times with 0.05 M...
phosphate-citrate-buffer (pH 5.0) before developing with 100 μg/ml 3,3′,5,5′-tetramethylbenzidine dihydrochloride in phosphate-citrate-buffer (pH 5.0) containing 0.006% H₂O₂. Finally, the reaction was stopped with 12.5 μl/well 1 N H₂SO₄ and the optical densities of the wells were read at 450 nm with an ELx800 microplate reader (Bio-Tek, UK). The coefficients of variance for interplate and interday variation of this ELISA were 4.2% and 4.6% respectively. The levels of serum MIP-1α and ICAM-1 were measured using commercial kits (DY270 DuoSet and DY720 DuoSet respectively, R & D Systems, UK) according to the manufacturer’s instructions.

**Statistical analysis**

The data were analyzed using Prism research software with Student’s t-test or Mann–Whitney U test, dependent on normality. A P value <0.05 was considered statistically significant.

Progression-free survival (PFS) was calculated from time of enrolment until disease progression or death last follow-up. Overall Survival (OS) was calculated from time of enrolment to death or last follow-up. Kaplan–Meier analysis was used to estimate PFS and OS. The log rank test assessed differences in progression or survival in patients with different immunological or clinical parameters.

**Results**

**Clinical Outcomes**

Twenty-two patients (21 male and one female) were enrolled in the study (Table 2). Patients received a median of 6 vaccines (range 1–12) with no significant local or systemic toxicity. Twenty-one patients were evaluable for response. One patient discontinued treatment before radiological disease reassessment. Four out of the 21 (19%) patients evaluable experienced an objective response as per RECIST 1.1 criteria. Two patients achieved a complete response (CR) and they are still alive at time of writing with an estimated overall survival of about 80 months for both. Seven patients had a stability of disease (SD). For five of these patients the disease remained stable for a period of 6 months or more for a total calculated clinical benefit (CB = CR + PR + SD ≥ 6 months) of 43% (9/21).

Upon closure of the trial, patients were retrospectively stratified into non-responder and responder based on the CB. Responders showed a significantly longer PFS (HR 0.23; 95% CI 0.08–0.62; P < .001) and OS (HR 0.22; 95% CI 0.08–0.59; P < .001; Figure 2). At the time of writing, 0/12 of the non-responders have survived however 3/9 of the responder group are alive.

**Serum Protein Analysis: Pre-Vaccination Differences in Responders and Non-Responders**

Pre-vaccination serum samples from the 12 clinically non-responding patients and 9 responding patients were quantified by means of a multiplex approach. We have analyzed 90 different serum proteins (Table 1) and results from the two different cohorts were compared.

We detected significant pre-vaccination differences between the non-responder population and the responder population for six proteins (Figure 1). Apolipoprotein C1 (86.2 μg/ml vs. 48.8 μg/ml; P = .05), IL-12 p40 (0.12 ng/ml vs. 0.02 ng/ml; P = .02), MIP1α (68.4 pg/ml vs. 52.1 pg/ml; P = .04), stem cell factor (259.8 pg/ml vs. 210.4 pg/ml; P = .02) and TNFα (6.1 pg/ml vs. 2.4 pg/ml; P = .03) levels were significantly increased in the non-responder group compared to the responders. In contrast to the elevation of these pro-inflammatory mediators seen in the non-responsive group of patients, a significant decrease in Lipoprotein A in the non-responders compared to responders (72.3 μg/ml vs. 163.2 μg/ml; P = .05) was observed. (See Fig. 4.)

No significant differences were observed between responders and non-responders for serum samples obtained at weeks 4 and 12. No significant changes were observed between baseline and samples at week 4 and 12.

**Validation of Markers Using ELISA and Predictive Techniques**

Since the low study numbers preclude the use of type I error correction in the cytometric bead data, we sought to identify additional methods to validate these data. Initially we used a powerful literature search software (Pathway studio) to develop a network of connectivity between the potential markers identified and then we attempted to find additional molecules that would fit into this network. These approaches led to the identification of ApoE as a putative additional marker. ApoE was quantified by ELISA and, as predicted, a significant difference between the non-responder and responder patients was observed (129.6 μg/ml responders vs. 201.5 μg/ml non-responders; P = .005; Figure 3). MIP1α and ICAM-1 were added as controls and ELISA data confirmed the original observations (MIP1α 42.0 pg/ml responders vs. 134.1 pg/ml non-responders; P = .03; ICAM 258.6 ng/ml responders vs. 286.9 ng/ml non-responders).
non-responders; \( P > .05 \); Figure 3). It must be noted that the ELISA and RBM microarray results differ in magnitude suggesting differences in the sensitivity of the two assays.

The mean value of ApoE observed in this cohort of subjects was selected to categorize patients as higher or lower ApoE serum level. Patients with levels of ApoE above the average had a significantly shorter PFS (HR 3.02; 95% CI 1.09–8.35; \( P = .015 \)) and OS (HR 2.40; 95% CI 0.9–6.3; \( P = .034 \)). No differences were observed for MiP1α.

**Neutrophil/lymphocyte Ratio and LDH**

No significant differences were observed in the neutrophil/lymphocyte ration and LDH levels over time nor between responders and non-responders (data not shown).

**Discussion**

Despite that the majority of patients do not gain any benefit from DC vaccines, this approach has occasionally given rise to dramatic responses in melanoma. Biomarkers are crucial to identify which patients are more likely to respond to DC vaccines. We looked for correlations between pre- or post- vaccination biomarkers and clinical outcomes to DC therapy in a cohort of patients with stage IV melanoma receiving an autologous DC vaccine. We have identified a general inflammatory marker profile and we validated the data using literature-searching software to predict for additional markers, which would be expected to be elevated if the original data is correct. We thus identified ApoE as an additional biomarker of non-response and we have subsequently confirmed this by ELISA.

ApoE has been the focus of intense interest with regard to immunomodulation as well as cancer for more than three decades. Despite that, there is still a lack of consensus on the role of ApoE in various cancers, overexpression of ApoE resulting in elevated levels in the serum, plasma or urine is associated with a poor prognosis or advanced stage in human cancers, including lung [8–10], gastric [11,12], ovarian [13,14] and bladder cancer [15,16]. On the contrary, ApoE was also shown to act as a direct metastasis-suppressive factor in melanoma [17], identifying ApoE as a potential biomarker for assessing tumor stage, metastasis, prognosis or response to treatment. Van den Elzen et al. proposed a pro-inflammatory role of ApoE, showing ApoE involved in the presentation of CD1a-loaded lipid antigens by antigen presenting cells (APC) for recognition by natural killer T (NKT) cells, which in turn respond by secreting cytokines (e.g. IFN-\( \gamma \)) and initiating an immune response [18]. A pro-inflammatory role of ApoE could also be due to its association with cell lipid homeostasis. ApoE possess lipophilic properties, potentially removing cholesterol and other lipids from the cell membrane and consequently initiating the recruitment of TLRs to lipid drafts as a
possible signal of cell damage [19]. As a result, transcriptional factors, such as NF-κB and interferon regulatory factors (IRFs) are activated and contribute to the production of pro-inflammatory cytokines [20,21]. Combined with additional markers, such as those identified in this study, measurement of ApoE could identify inappropriate inflammation in patients prior to treatment and thus identify patients who are most unlikely to benefit from DC immunotherapy.

Inflammation in cancer may be caused by tumor invasion and this perturbation of homeostasis could generate a sterile inflammatory response [22]. Intuitively inflammation would seem to be prerequisite for activation of the immune response and the generation of an anti-tumor outcome. However, chronic inflammation has a negative correlation with cancer outcomes [23,24]. The immune response status at the time of diagnosis may have a bigger impact on prognosis than the typical staging procedures irrespective of other treatments given [25].

One possible mechanism for the influence of inflammation on cancer is the shift between a desirable Th1 (cell mediated immunity associated) response and a non-effective Th2 (humoral immunity associated) response [26]. If this were the case, then one clinical possibility would be to pre-treat patients about to undergo immunotherapy with an anti-inflammatory protocol.

It is interesting to note that there are several reports, which have observed an improved response to vaccination in mice when they have been pre-treated with known anti-inflammatories. At present, the strongest evidence available that confirms pre-treating is beneficial has been shown with Lenalidomide [27,28]. This was subsequently confirmed in humans with multiple myeloma patients who responded to Pevnlar after they had been pre-treated with Lenalidomide [29]. Although Lenalidomide has been reported as having a co-stimulatory function, it is also a strong inhibitor of inflammation through the Cox-2 pathways [30]. Hence, a pre-immunotherapy course of anti-inflammatory treatment might render the potentially unresponsive patient clinically responsive. In this study we have described a panel of potential inflammatory markers that can help to identify patients less likely to respond to a DC-vaccine. Other groups have looked at larger cohorts of patients treated with PD-1 inhibitors or high dose IL-2 and they have detected similar biomarkers. In keeping with our findings, they are present prior to treatment and rather than as a result of treatment, they are elevated in non-responders and include many markers of chronic inflammation as described here [31,32].

**Conclusions**

In summary, our findings support the notion that treating the inflammatory background may have an impact on clinical outcome for patients receiving immunotherapy. A larger study is needed to confirm the significance of ApoE as a predictive biomarker for response to DC vaccines.

**Declarations**

**Ethics Approval and Consent to Participate**

The study was approved by the Local Ethics Committee at St George’s Hospital Medical School (Ethics Committee reference number: 03.0285). Study participants signed informed consent prior to study participation.

**Consent for Publication**

This manuscript does not contain individual data presented in a manner that could identify them.

**Availability of Data and Materials**

Data can be released in conjunction with study participant signed consent form. The datasets analyzed during the current study are not publicly available given the restrictions arising from the signed consent.

**Author Contributions**

AGD was the PI of the clinical trial and supervised the whole project. Experiments were designed by MBS and carried out by EK and AG under the supervision of MBS, GC and JC. The ApoE ELISA was developed by KBS. HL and DG acquired the clinical data. Analysis of the clinical data was carried out by AF and AGD. The paper was written by MBS, AF and AGD with contributions from HL, AG and JC.

All Authors read and approved the manuscript.

**Acknowledgements**

Thank you to Nikki Read for analyzing the serum samples for ApoE as part of her undergraduate project. The Authors would like to thank the Fischer Family Trust and the Institute for Cancer Vaccines and Immunotherapy (ICVI) for funding the study.
References

[1] Rosenberg SA, Yang JC, and Restifo NP (2004). Cancer immunotherapy: moving beyond current vaccines. Nat Med 10, 909–915.

[2] Higano CS, Schellhammer PF, Small EJ, Burch PA, Nemunaitis J, Yuh L, Provost N, and Frohlich MW (2009). Integrated data from 2 randomized, double-blind, placebo-controlled, phase 3 trials of active cellular immunotherapy with sipuleucel-T in advanced prostate cancer. Cancer 115, 3670–3679.

[3] Chua TC, Chong CH, Liaw W, Zhao J, and Morris DL (2012). Inflammatory markers in blood and serum tumor markers predict survival in patients with epithelial appendiceal neoplasms undergoing surgical cytoreduction and intraperitoneal chemotherapy. Annu Surg 256, 342–349.

[4] De Raaf PJ, Steijger S, Lamers CH, Jager A, Gratama JW, and Van Der Rijt CC (2012). Inflammation and fatigue dimensions in advanced cancer patients and cancer survivors: an exploratory study. Cancer 118, 6005–6011.

[5] Lippitz BE (2013). Cytokine patterns in patients with cancer: a systematic review. Lancet Oncol 14(6), e218–e228.

[6] John J, Hutchinson J, Dalgleish A, and Pandha H (2003). Cryopreservation of immature monocyte-derived dendritic cells results in enhanced cell maturation but reduced endocytic activity and efficiency of adenosine transduction. J Immunol Methods 272, 35–48.

[7] Kovalcsik E, John J, Turner M, Birchall L, Sage D, Whittle R, Dalgleish A, and Pandha H (2004). Differential expression of melanoma-associated antigens and molecules involved in antigen processing and presentation in three cell lines established from a single patient. Melanoma Res 14, 463–471.

[8] Luo J, Song J, Feng P, Wang Y, Long W, Liu M, and Li L (2016). Elevated E expression promotes lung adenocarcinoma proliferation and migration and as a potential survival marker in lung cancer. Tumour Biol 37, 10715–10721.

[9] Su WP, Chen YT, Lai WW, Lin CC, Yan JJ, and Su WC (2011). Apolipoprotein E expression promotes lung adenocarcinoma proliferation and migration and as a potential survival marker in lung cancer. Lung Cancer 71, 28–33.

[10] Wang Y, Chen Z, Chen J, Pan J, Zhang W, Pan Q, Ding H, Lin X, Wen X, and Li Y, et al (2013). The diagnostic value of apolipoprotein E in malignant pleural effusion associated with non-small cell lung cancer. Clin Chim Acta 421, 230–235.

[11] Sakashita K, Tanaka F, Zhang X, Mimori K, Kamohara Y, Inoue H, Sawada T, Hirakawa K, and Mori M (2008). Clinical significance of ApoE expression in human gastric cancer. Oncol Rep 20, 1313–1319.

[12] Shi X, Xu J, Wang J, Cui M, Gao Y, Niu H, and Jin H (2015). Expression analysis of apolipoprotein E and its associated genes in gastric cancer. Oncol Lett 10, 1309–1314.

[13] Chen YC, Pohl G, Wang TL, Morin PJ, Risberg B, Kristensen GB, Yu A, Davidson B, and Shih Ie M (2005). Apolipoprotein E is required for cell proliferation and survival in ovarian cancer. Cancer Res 65, 331–337.

[14] Poersch A, Grassi ML, Carvalho VP, Lanfredi GP, Palma Cde S, Greene LJ, de Sousa CB, Carrara HH, Candido Dos Reis FJ, and Faca VM (2016). A proteomic signature of ovarian cancer tumor fluid identified by high throughput and verified by targeted proteomics. J Proteomics 145, 226–236.

[15] Linden M, Lind SB, Myhrholer C, Segersten U, Wester K, Lyutvinskiy Y, Zubaner R, Malmstrom PU, and Pettersson U (2012). Proteomic analysis of urinary biomarker candidates for nonmuscle invasive bladder cancer. Proteomics 12, 135–144.

[16] Zhang G, Gomes-Giacoia E, Dai Y, Lawton A, Miryake M, Furuya HS, Goodison S, and Rossor CJ (2014). Validation and clinicopathologic associations of a urine-based bladder cancer biomarker signature. Diagn Pathol 9, 200.

[17] Pencheva N, Tran H, Buss C, Huh D, Drobnjak M, Busam K, and Tavazoe SF (2012). Convergent multi-miRNA targeting of ApoE drives LRPI/LRP8-dependent melanoma metastasis and angiogenesis. Cell 151, 1068–1082.

[18] van den Elzen P, Garg S, Leon L, Brigl M, Leadbetter EA, Gumperz JE, Dascher CC, Cheng TY, Sacks FM, and Illarionov PA, et al (2005). Apolipoprotein-mediated pathways of lipid antigen presentation. Nature 437, 906–910.

[19] Morrison C, Baer MR, Zandberg DP, Kimball A, and Davila E (2011). Effects of Toll-like receptor signals in T-cell neoplasms. Future Oncol 7, 309–320.

[20] Endo Y, Blinova K, Romantseva T, Golding H, and Zaitseva M (2014). Differences in PGE2 production between primary human monocytes and differentiated macrophages: role of IL-1beta and TRIF/IRF3. PLoS One 9:e95817.

[21] Yu L, Wang L, and Chen S (2010). Endogenous toll-like receptor ligands and their biological significance. J Cell Mol Med 14, 2592–2603.

[22] Hirsiger S, Simmen HP, Werner CM, Wanner GA, and Rittirsch D (2012). Danger signals activating the immune response after trauma. Mediators Inflamm 2012, 315941.

[23] O’Byrne KJ and Dalgleish AG (2001). Chronic immune activation and inflammation as the cause of malignancy. Br J Cancer 85, 473–483.

[24] Dalgleish AG and O’Byrne KJ (2002). Chronic immune activation and inflammation in the pathogenesis of AIDS and cancer. Adv Cancer Res 84, 231–276.

[25] Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pages C, Tosolini M, Camus M, Berger A, and Wind P, et al (2006). Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 313, 1960–1964.

[26] Lucey DR, Clerici M, and Shearer GM (1996). Type 1 and type 2 cytokine dysregulation in human infectious, neoplastic, and inflammatory diseases. Clin Microbiol Rev 9, 532–562.

[27] Dredge K, Marriott JB, Todryk SM, Muller GW, Chen R, Stirling DI, and Dalgleish AG (2002). Protective antitumor immunity induced by a costimulatory thalidomide analog in conjunction with whole tumor cell vaccination is mediated by increased Th1-type immunity. J Immunol 168(10), 4914–4919.

[28] Bartlett JB, Dredge K, and Dalgleish AG (2004). The evolution of thalidomide and its IMiD derivatives as anticancer agents. Nat Rev Cancer 4, 314–322.

[29] Noonan K, Rudraraju L, Ferguson A, Emerling A, Pasetti MF, Huff CA, and Borrello I (2012). Lenalidomide-induced immunomodulation in multiple myeloma: impact on vaccines and antitumor responses. Clin Cancer Res 18, 1426–1434.

[30] Nguyen-Pham TN, Jung SH, Vo MC, Thanh-Tran HT, Lee YK, Lee HJ, Choi NR, Hoang MD, Kim HJ, and Lee JJ (2015). Lenalidomide synergistically enhances the effect of dendritic cell vaccination in a model of murine multiple myeloma. J Immunother 38, 330–339.

[31] Sullivan RJ, Logan T, Khushalani N, Margolin K, Koon H, Olencki T, Hutson T, Curi R, Boder J, and Blackmon S, et al (2016). Application of a test developed for prediction of response to high dose interleukin-2 (HDIL-2) and the BDX008 test for prediction of outcomes following checkpoint inhibitors to cohorts of patients treated with HDIL-2 or nivolumab. SITC 2016 Poster 106; 2016.

[32] Weber JS, Sznol M, Sullivan RJ, Blackmon S, Beland K, Kluger HM, Halaban R, Bacchiocchi A, Ascieto PA, and Capone M, et al (2018). A Serum protein signature associated with outcome after anti–PD-1 therapy in metastatic melanoma. Cancer Immunol Res 6, 79–86.