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REVIEW **Open Access**

Immunology of tissue homeostasis, ovarian cancer growth and regression, and long lasting cancer immune prophylaxis - review of literature

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Summary. Data on the substantial physiological role of the immune system in the organism's ability to manage proper differentiation and function of normal tissues (tissue homeostasis), and detailed causes of the immune system's essential role for the *in vivo* stimulation of cancer growth, are severely lacking. This results in a lack of effective cancer immunotherapy without adverse events, and in the lack of long-lasting cancer immune prophylaxes, particularly in ovarian cancers. Elimination of blood auto-antibodies blocking anti-cancer T cell effectors by intermittent moderate doses of cyclophosphamide, facilitation of the immune system reactivity against alloantigens of cancer cells by two subsequent blood transfusions, and augmentation of anticancer immunity by weekly intradermal injections of bacterial toxins, caused during the subsequent treatment-free period, lasting for two to four weeks, regression of inoperable epithelial ovarian cancers and regeneration of the tremendously metastatically altered abdominal tissues into normal healthy conditions without multivisceral cytoreductive surgery, which can result in life-threatening consequences. An otherwise untreated rectal cancer, progressing over 3 years, regressed after severe toxic dermatitis lasting over one week. This was caused by an accidental consumption of a large raw shiitake mushroom. Subsequent daily consumptions of 2 g Metformin ER and honeybee propolis ethanol extract, and weekly single larger raw shiitake mushroom, which all stimulate immune system reactivity against cancer stem cells, prevented malignant recurrence over the next 29 years without recurring dermatitis, and maintained healthy organism's conditions. These observations indicate that regression of advanced inoperable cancers and long-lasting cancer immune prophylaxis can be reached by simple approaches.

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Introduction

Cancer immunotherapy is a treatment that uses a patient's own immune system to help fight cancer and, as such, it has several advantages over other treatments. Major milestones in immunotherapy came in the middle of the 1980s as a) adoptive cell therapy relying on patients' tumor infiltrating lymphocytes, b) injection of recombinant cytokines such as rIL2, c) identification of the first tumor associated antigens, and d) development of tumor specific monoclonal antibodies. It was followed by dendritic cell vaccines. Tremendous progress has been made in the past two and half decades with regard to understanding the complex interactions between tumors and the immune system and developing innovative ways to manipulate the anti-tumor immune response. It was recently represented as blockage of immune checkpoint inhibitors (Rihova and Stastny, 2015).

However, immune-related adverse events, ranging from mild to lethal, have been found to accompany immunotherapy with checkpoint inhibitors, but their underlying causes are not well understood (Editorial, 2017). Review of 22 clinical trials (1265 patients) with anti-CTLA-4 treatment indicated that the overall incidence of adverse events was 72%, with high grade 24%, and death occurring in 0.86% of patients (11 cases). Adverse events included inflammation of gastrointestinal tract, skin lesions (rash, pruritus, and vitiligo), hepatitis, hypophysitis, thyroiditis, sarcoidosis, uveitis, Guillain-Barre syndrome, immune-mediated cytopenia and polymyalgia rheumatic (Bertrand et al.,

Abbreviations. ASCD, asymmetric stem cell division; CSC, cancer stem cell; DCD, differentiating cell daughter; EOC, epithelial ovarian cancer; MDC, monocyte-derived cell; pMDC, primitive MDC; SCD, Stem cell daughter; STC, suicidal T cell

2015). Adverse events altering the quality of life can also be caused by common cancer chemotherapy - nausea, vomiting, constipation, diarrhea, fatigue, hair loss, and mucositis (Lotfi-Jam et al., 2008). Platinum-based chemotherapy may cause neurotoxicity and result in chronic debilitation (McWhinney et al., 2009). Besides that, the survival of women with epithelial ovarian cancers (EOCs) has remained unsatisfactory during the last several decades (Pisano et al., 2009; Weiderpass and Tyczynski 2015). Novel therapies without adverse events (Papa et al., 2016) and prevention of cancer recurrences are needed to improve treatment and long-lasting survival of patients with EOCs.

Immune system and tissue homeostasis

For effective cancer immunotherapy without adverse events, we need to better understand details of the immune system niche involvement in the homeostasis of all body tissues and the host immune niche support of cancer growth *in vivo*. T cells contribute to the asymmetric stem cell division (ASCD) of tissues during their regeneration. Along with monocyte-derived cells (MDCs), they also stimulate differentiation of postmitotic cell daughters into functional stages, the extent of which differs between certain tissue types, depending on the course of particular tissue differentiation during prenatal developmental immune adaptation (Bukovsky, 2011). The immune system belongs to a complex tissue control system (Bukovsky et al., 1983), which includes vascular pericytes that accompany postcapillary venules. Pericytes stimulate the earliest differentiation of postmitotic tissue cells and proliferating endothelial cells, and their activity is regulated by perivascular autonomic innervation that controls tissue quantity (Bukovsky et al., 1991, 2001a). Autonomic innervation is absent in neovascularized primary (Terada and Matsunaga, 2001) and metastatic (Ashraf et al., 1996) malignant tissues, where vascular pericytes exhibit extreme activity resulting in unlimited expansion of cancer cells and progressive cancer bulk vascularization (Bukovsky, 2016). Intraepithelial lymphocytes are permanently present in the skin, intestine, biliary tract, oral cavity, lungs, upper respiratory tract, and reproductive tract tissues (Lambolez et al., 2013). The lymphocytes are also present in the intermediate lobe of the pituitary gland (Shanklin, 1951) and in liver sinusoids (Winnock et al. 1995), and they regulate thyroid hormone activity (Klein, 2006). T cells play a role in local immune function and in differentiation of epithelial cells (Barrett et al., 1992), and contribute to epithelial homeostasis (Komori et al., 2006; Hirosako et al., 2009; Macleod and Havran, 2011).

Basic units of the tissue control system are associated with postcapillary venules (Figs. 1, 2), where primitive MDCs (pMDCs) interact with endothelial and tissue stem cells to enable their asymmetric stem cell division (ASCD), if functionally required (Bukovsky at al., 2001a). The ASCD requires *in vivo* involvement of suicidal T cells (STC) giving rise to new daughter stem cells (SCDs) and differentiating cell daughters (DCDs) (Bukovsky, 2016). Suicidal lymphocytes have been identified in dividing cells of the mouse intestinal epithelium (Andrew and Andrew, 1945), and CD8+ STCs were identified during ASCD of ovarian stem cells within emerging new germ cells in adult human ovaries (Bukovsky, 2015). Host suicidal CD8+ T cells are also essential for the asymmetric division of perivascular cancer stem cells (CSCs) - see Fig. 5 below.

The immune system has been considered to provide organism protection against nonself substances and ignore self tissues due to the elimination of autoreactive lymphoid cells (Conrad et al., 2007; Mueller 2010). This is an incorrect assumption for two reasons: T cells are required for the proper function of some body tissues (see above), and autoimmunity is a consequence of an altered "stop effect" of pMDCs, either due to the altered tissue development during the prenatal immune adaptation (e.g. type 1 diabetes) (Bukovsky, 2011), or due to age-induced regression of the immune system function (Mathe, 1997), which causes age-associate diseases in certain tissues. e.g. Alzheimer's disease (Bukovsky, 2011, 2016).

The CD8+ T cells play an important role in homeostasis of epithelial tissues by enabling as STCs the ASCDs of epithelial and other tissues stem cells *in vivo*, and also stimulate early differentiation of regenerating tissue cells. While exhibiting gradual decline among differentiating tissue cells, the CD8+ T cells also transform MDCs into dendritic cells (Bukovsky, 2016). Fig. 2G shows that the T cells accumulate among basal epithelial stem cells, where they participate in the stimulation of stem cell ASCDs. They release CD8 among early differentiating parabasal cells, interact with MDCs to stimulate their transformation into intraepithelial dendritic cells, and degenerate. The intraepithelial MDCs release HLA DR protein among moderately differentiated parabasal cells, and dendritic cells regress among moderately differentiated cells in the intermediate epithelial layer. Hence T cells and MDCs interact and degenerate to stimulate regeneration and maturation of epithelial cells (Bukovsky, 2011). The regenerative renewal interval for intestinal cells is 2-4 days, 6 days in the uterine ectocervix, 10-30 days for epidermal cells, and 6 to 12 months for liver hepatocytes (Milo and Phillips, 2015). Therefore, the presence of suicidal CD8 T cells among tissue stem cells is more common in tissues with fast cellular regeneration, compared to the slower ones, e.g., intestinal cells vs. hepatocytes. During regeneration of the liver after partial hepatectomy, however, numerous T cells emerge to stimulate hepatocyte renewal, until the normal liver volume reappears (Cuk et al., 1987).

CTLA-4 is expressed by both CD4 and CD8 T cells (Nakamoto et al., 2009). Therefore, the immune-related

adverse events accompanying CTLA-4 blocking will predominantly affect tissues where T cells are involved in the stimulation of tissue regeneration by ASCD and in differentiation of postmitotic cells into the functional stages (compare the tissues altered by adverse events and permanent presence of T cells in tissues listed above). On the other hand, tissues lacking a permanent presence of T cells, like the brain, skeletal muscles or pancreas, are not as likely to be affected by CTLA-4 blocking. Their regeneration, however, will be altered, since it requires involvement of CD8+ STCs in the stimulation of ASCD by stem cells.

How to treat severe immune-related adverse events

It is apparent that adverse immune events after

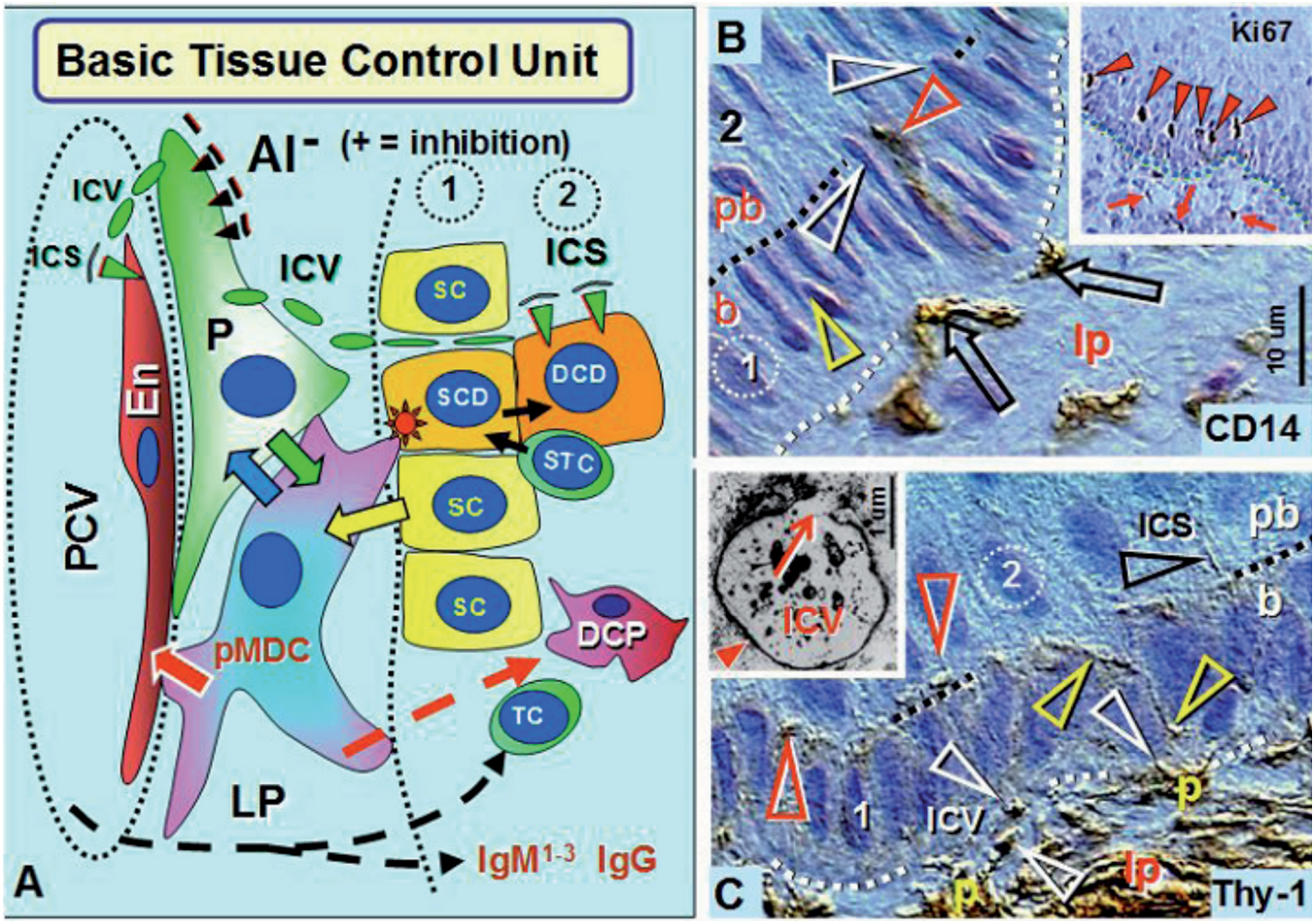

Fig. 1. Basic tissue control unit and early differentiation of postmitotic tissue cells. **A.** Basic tissue control unit (TCU) is associated with postcapillary venule (PCV). The TCU consists of CD14+ primitive MDCs (pMDC), pericytes (P) accompanying PCV, and autonomic innervation (AI) that controls pericytes and TCU activity to manage the proper tissue quantity. The pMDCs are dominant components of the TCUs. They receive signals (yellow arrow) to regenerate from tissue stem cells (SC) when functionally required, and communicate with pericytes (blue arrow) for their support. If if received (green arrow), the pMDCs will stimulate asymmetric stem cell division (red asterisk), which is accompanied by a suicidal T cell (STC). This gives rise to the stem cell daughter (SCD) and differentiating cell daughter (DCD). The pericytes stimulate by Thy-1+ intercellular vesicles (ICV) growth factors and cytokines differentiation of postmitotic DCDs and also to divided endothelial cells, e.g., during the growth of ovarian follicles. After release of ICV content (green arrowheads), the ICVs collapse into intercellular spikes (ICS). The pMDCs also communicate with endothelial cells (red arrow) to regulate influx of tissue-committed T cells (TC) and immunoglobulins (IgM1-3, IgG), depending on the pMDC properties outlined for the particular tissue during prenatal immune adaptation. Accordingly, some pMDCs transform into dendritic cell precursors (DCP). **B.** Some of the pMDCs in the ectocervical epithelium lamina propria (LP) enter (arrows) basal epithelial layer (b), interact with basal epithelial stem cells (yellow arrowhead), and migrate to the inner parabasal layer (pb; red arrowhead). Postmitotic basal cells (white arrowheads) migrate to the parabasal layer. Inset: Arrowheads indicate Ki67+ postmitotic parabasal DCDs (note a lack of Ki67 expression in the corresponding basal stem cell daughters); arrows indicate postmitotic DCDs in the peri-vascular lamina propria. **C.** Thy-1+ pericytes (p) in the ectocervical lamina propria produce intercellular vesicles (white arrowheads), which migrate through the basal layer (yellow arrowheads) to the postmitotic parabasal cells (red arrowheads) to empty their content and transform into intercellular spikes (black arrowhead). Inset shows Thy-1 immunolabelling in the transmission electron microscopy of the intercellular vesicle that is releasing its content (arrow). Adjusted from (Bukovsky at al 2001a; Bukovsky 2011): ©Antonin Bukovsky.

checkpoint blockades alter those tissues where committed T cells are required for their proper homeostatic function. It is possible that severe multiorgan adverse events, which may cause the patient's death, can be treated by blood transfusions from young healthy donors of the same sex and ethnicity, as proposed for the treatment of age-associated diseases (Bukovsky, 2016). This can cause functional repair of tissues requiring committed T cells for regeneration by ASCD from their stem cells and for differentiation of DCDs into the functional stages. The effect of such treatment in affected patients can last till the regeneration of the recipient's own immune system homeostatic effectors regulating the regeneration and function of dependent tissues, such as blood mononuclear cells, have been found to survive in trauma-affected blood recipients for 6 to 18 months (Lee et al., 1999) or longer, due to the transfusion-associated microchimerism (Lee et al., 2005; Bloch et al., 2013). This should, however, be accompanied by an effective cancer treatment that is accompanied by blood transfusions, as reported below.

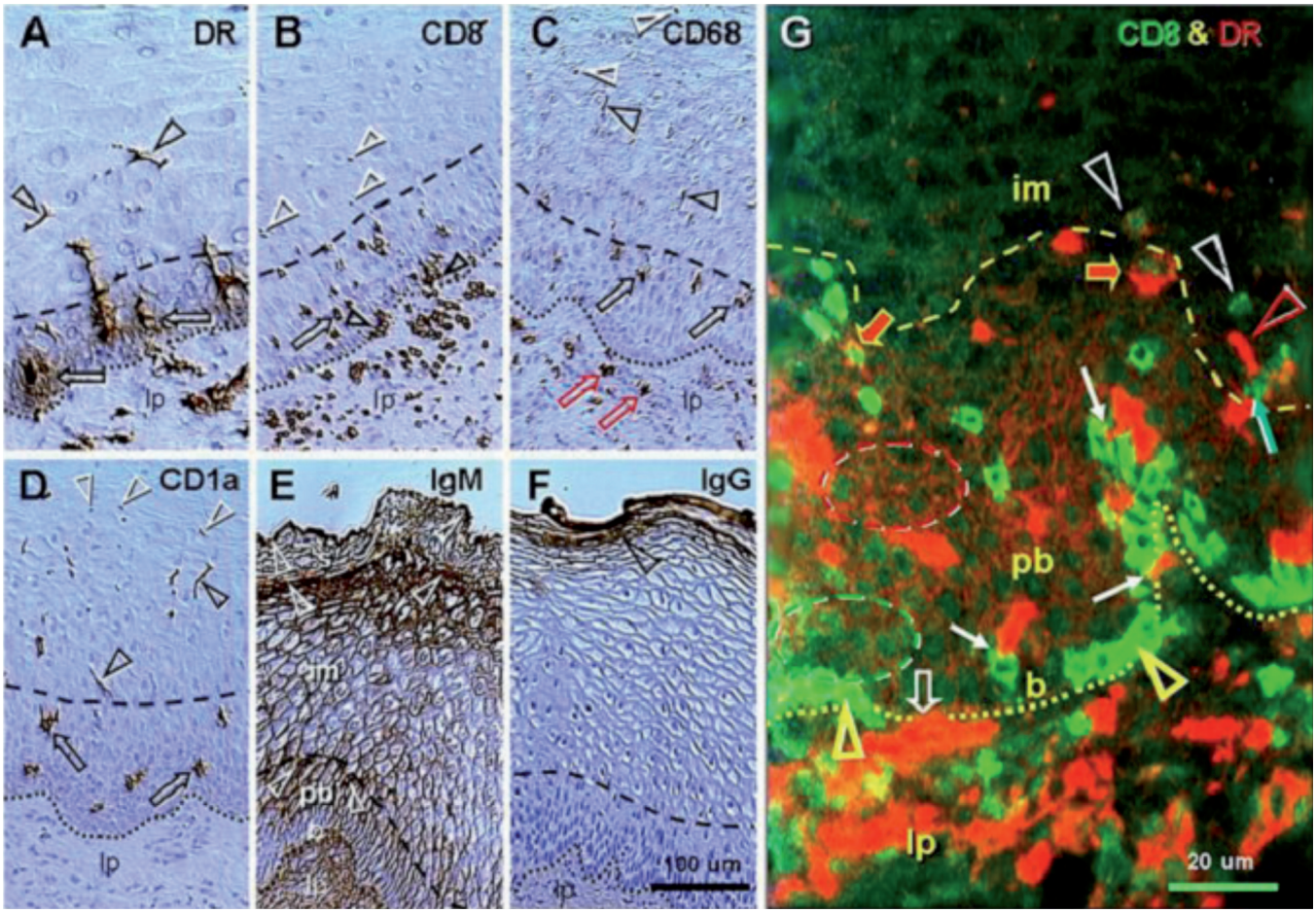

Fig. 2. Differentiation and regression of MDC-derived cells and T cells, and binding of IgM and IgG in the squamous epithelium of the uterine ectocervix. **A.** HLA-DR+ MDC release DR molecules in the parabasal epithelial layer (arrows) and transform into dendritic cells in the intermediate layer (arrowheads). **B.** CD8 T cells migrate from lamina propria into the basal (black arrowheads) and parabasal (arrow) layers and undergo apoptosis after entering the intermediate layer (white arrowheads). **C.** The MDCs express CD68 in the lamina propria (red arrows) and upper parabasal layer (black arrows), and CD68+ dendritic cells (black arrowheads) undergo apoptosis in the mid intermediate layer (white arrowheads). **D.** CD1a stains MDC in the parabasal layer (arrows) and dendritic cells in a similar manner as CD68. Note a lack of CD1a expression in the lamina propria. **E.** IgM binds to the upper parabasal, intermediate and superficial layers (arrowheads). **F.** IgG bins to the entire superficial layer. **G.** CD8+ T cells (green) accumulate (yellow arrowheads) in the basal layer (b) and DR+ MDC (red) entering epithelium (open arrow) interact with T cells in the basal and parabasal (pb) layers (solid white arrows). At the parabasal/intermediate interface (white dashed line), the T cells express DR (red arrows) indicating their activation. The T cells entering the intermediate layer (green arrow) cause differentiation of MDC into dendritic cells (red arrowhead) and degenerate (white arrowheads). Green dashed line ellipse indicates CD8 protein release from T cells among early differentiating parabasal cells, and red dashed line ellipse indicates DR release from MDCs among moderately differentiated parabasal cells. Adjusted from (Bukovsky et al., 2001a; Bukovsky, 2011): ©Antonin Bukovsky.

Why do cancer cells grow in immunocompetent mammalian organisms?

The growth of epithelial cancers has been suggested to be due to the ability of cancer cells to utilize conditions similar to those required for survival of semiallogeneic embryos in mammalian pregnancies (Bukovsky et al., 2001b). Human and mouse cancer cells express allogeneic class I and class II MHC determinants (Lindahl, 1979; Ferrone et al., 1980). Hence, host support of cancer growth is comparable to the support of semiallogeneic (or allogeneic in the donor egg recipients) mammalian embryo implantation and growth. During implantation, the embryonic trophoblast hybridizes with the uterine epithelium (Larsen, 1970). Feto-placental vessels lack innervation, hence control of this circulation is dependent on both locally produced and circulating vasoactive factors (Buttery et al., 1994). The difference between blood vessels in tumors and normal tissues has been recognized for a long time. Tumor endothelium proliferates 20 to 2000 times faster than that of any normal tissue endothelium in adult individuals, except in the placenta, which has an even faster endothelial proliferation (Denekamp, 1984).

Host support of epithelial ovarian cancer growth

Cells of epithelial cancers express macrophage antigens (Bukovsky at al., 2001b; Shabo and Svanvik, 2011) due to hybridization with the host pMDCs (Bukovsky, 2016) - (Fig. 3). Immunohistochemical study of EOCs demonstrates staining for MDC markers in cancer cells adjacent to the host microvasculature, except for their stem cells (Fig. 4A,C,D,E). Dashed line ellipses in Fig. 4B show Ki67 expression by pairs of nuclei in dividing cancer cells distant from the cancer microvasculature. The observations above (see inset in Fig. 1B) indicate that Ki67 is expressed by postmitotic DCDs, but not by the corresponding SCDs. However, pairs of nuclei in spermatogonial and oogonial stem cells undergoing meiosis I symmetric division (cytokinesis) exhibit strong Ki67 expression in monkey testes and ovaries (Yuan et al., 2013). Therefore, Ki67 staining of nuclear pairs in Fig. 4B indicates presence of developing haploid malignant cells formed during the meiosis I of ovarian stem cells (Bukovsky, 2015). The generation of the haploid cells in meiosis I was supported by cancer/testis SCP-1 protein that was identified in malignant gliomas, and in breast, renal cell, and ovarian cancers. SCP-1 has been proposed to contribute to genomic instability of such malignant cells (Tureci et al., 1998). The occurrence of cancer cell divisions at the surface of the EOC tumor bulk contributes to their release into pelvic and abdominal cavities and in the common development of EOC metastases. The left ovarian vein often drains into the left renal vein (Mantha et al., 2015) often contributing the liver veins, and perivascular CSCs released into the postcapillary venules can often cause liver metastases.

Fig. 4E (detail from panel D) shows membrane apposition between the cancer DCD and perivascular host MDC during the formation of the cancer DCD/host MDC hybrid (Fig. 3). Hybridization with the host pMDCs allows cancer cells to utilize the dominant role of pMDC in the management of tissue homeostasis and vascular growth (see Fig. 1 legend). Also shown is a dividing unstained CSC exhibiting denser cytoplasm in one of its developing daughters (red arrow). The inset in Fig. 5A shows dividing CSC containing CD8+ STC in one of the emerging cancer cell daughters, resembling the situation during ASCD in the adult human ovary (Bukovsky, 2015). The cancer DCDs containing STCs

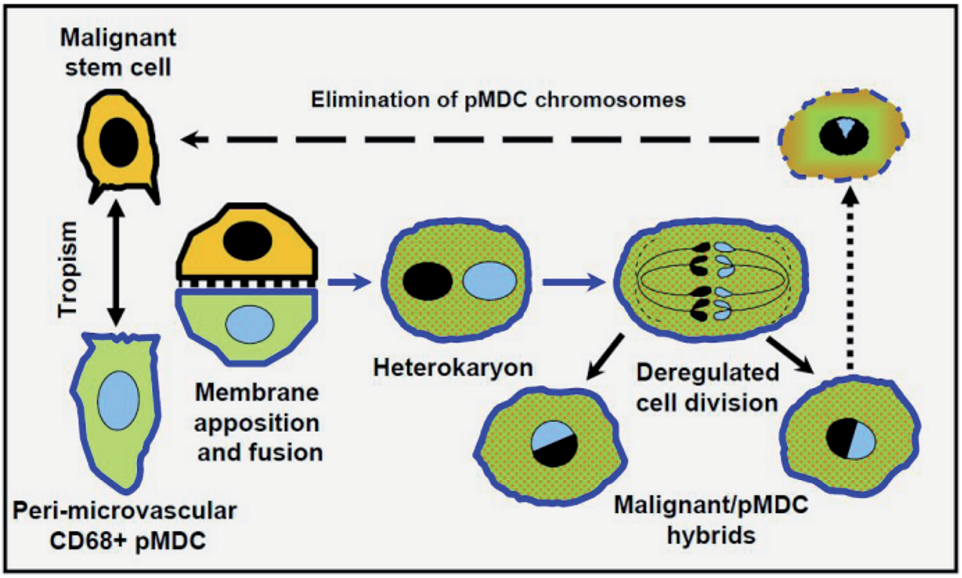

Fig. 3. Hybridization of malignant stem cells with the host peri-microvascular pMDCs. Scheme of the fusion between cancer stem cells and peri-microvascular MDCs that is causing the development of hybrids with surface expression of MDC markers and cytoplasmic preservation of the malignant potential. Occasional elimination of normal chromosomes results in hybrid reversal back to the malignant stem cell. Adjusted from (Bukovsky, 2011): ©Antonin Bukovsky.

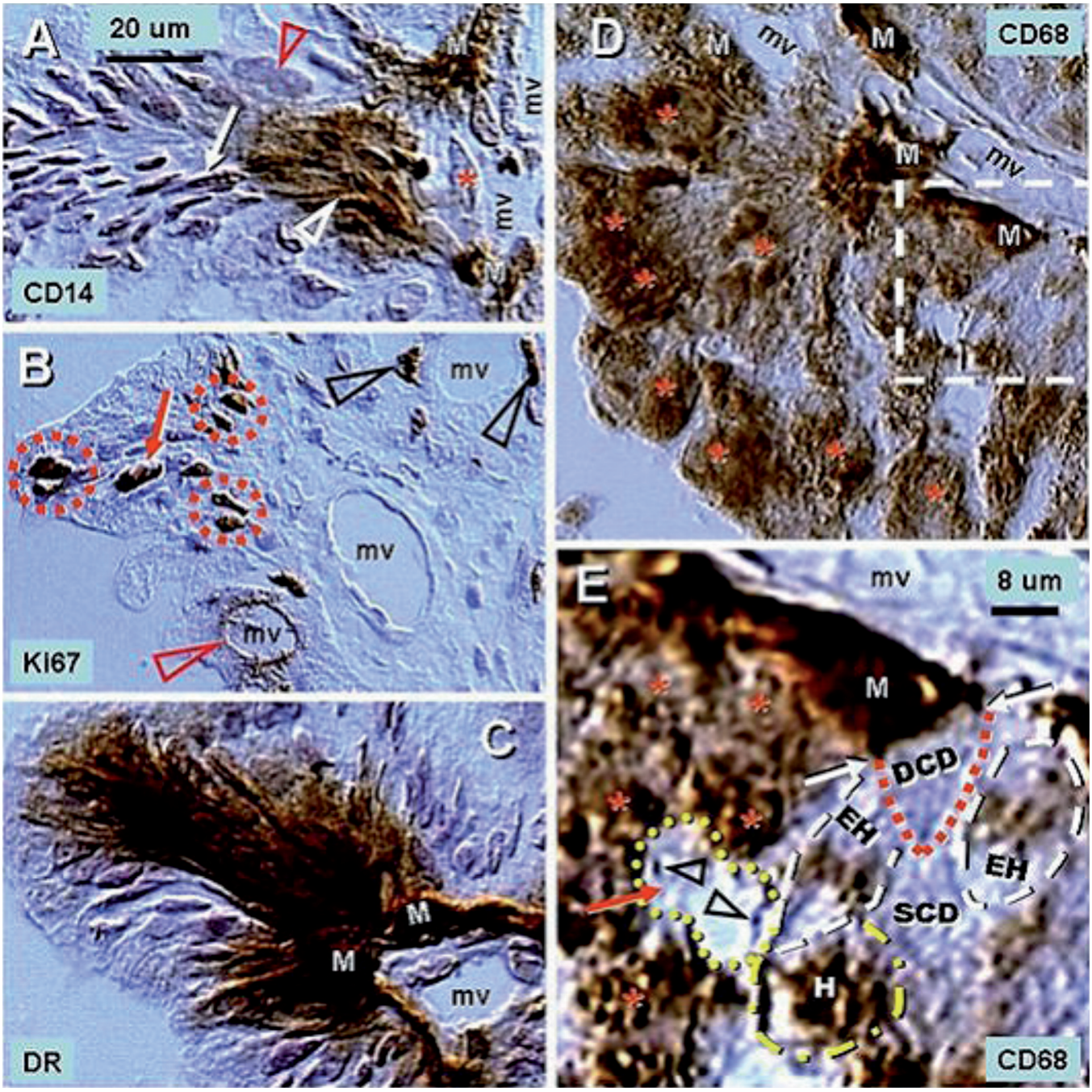

Fig. 4. Expression of pMDC markers by cancer cells, formation of haploid cancer cells at tumor surface, and process of hybridization with host pMDCs in the advanced EOCs. **A.** Cancer microvasculature (mv) is accompanied by unstained CSC (red asterisk) and CD14+ MDCs. Adjacent cancer/MDC hybrids are densely stained (white arrowhead). More distant are cancer cells showing regression of cytoplasmic staining (white arrow), and some of them lose CD14 marker and revert back into dividing unstained CSCs (red arrowhead). **B.** Parallel section shows microvasculature accompanied by Ki67+ postmitotic MDCs (black arrowheads), proliferating vascular endothelium (red arrowhead). Distant cancer cells exhibit Ki67+ pairs of nuclei (red dashed line ellipses) durinng their meiosis I division (see text). Red arrow indicates Ki67+ postmeiotic cancer cell migrating to the cancer bulk surface. **C.** Parallel section shows marked DR staining of perivascular pMDC (M). Note marked DR expression by adjacent cancer cells/MDCs hybrids. The distant cancer cells are unstained and some of them represent Ki67+ post-meiotic cancer cells (see panel **B**). **D.** The CD68 is expressed on perivascular MDCs (M). Cytoplasmic and surface staining is abundant on the cancer cells (red asterisks) in the tumor mass. **E.** Detail from panel **D** (see dashed line in panel **D** shows unstained postmitotic CSC daughters represented by the stem cell daughter (SCD) and "differentiating" cell daughter (DCD). The DCD (dotted red line) associates with a perivascular pMDC during initiation of cancer cell hybridization by membrane apposition (white arrows). Two previously formed early hybrids (EH) originating from deregulated cell division of the binuclear hybrid cell (heterokaryon in Fig. 3), show perinuclear CD68 staining only. More advanced hybrid (H) shows almost complete cytoplasmic expression of CD68 MDC marker. Unstained dividing CSC (yellow dotted line) is in telophase with reappearance of the nucleoli (arrowheads). Red arrow indicates more dense cytoplasm in one of the emerging daughter cells - compare with the presence of intracytoplasmic CD8+ T cell in the inset of Fig. 5A and cancer "differentiating" cell precursor (cCDCp) containing the vital host STC in Fig. 5C. Adjusted from (Bukovsky, 2011, 2016): ©Antonin Bukovsky.

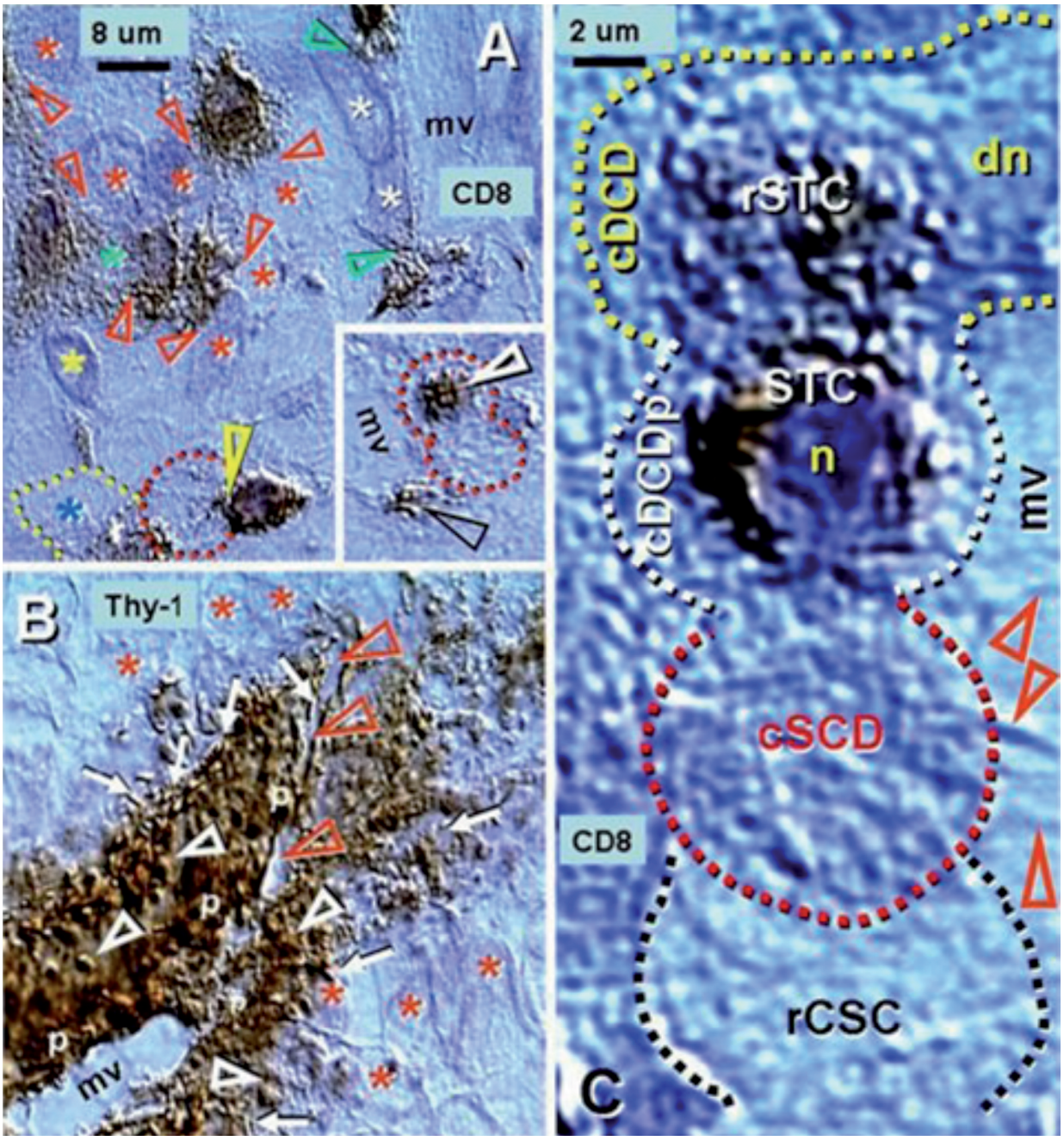

Fig. 5. The perivascular suicidal and tumor infiltrating CD8 T cells, marked activity of pericytes in the advanced EOCs, and T cell involvement in the asymmetric division of cancer stem cell. Panel **A** shows host CD8+ STCs interacting (green arrowheads) with peri-microvascular (mv) CSCs (white asterisks) to stimulate their ASCDs. The CD8 T cells also interact (red arrowheads) with more distant cancer cells (red asterisks) and release CD8 protein to stimulate their early growth (green asterisk), like in the normal tissues (see dashed green ellipse in Fig. 2G). Yellow arrowhead indicates CD8+ T cell entering CSC (red dotted circle) and yellow dotted line and blue asterisk indicate cancer DCD containing regressing CD8 T cell (white r). Inset shows asymmetric division of perivascular CSC marked with red dotted line. The cytoplasm of one daughter cell is occupied by the host CD8+ STC (white arrowhead). The occupied daughter cell is expected to contribute as DCD to the larger cancer cells in the tumor mass (see yellow asterisk in panel **A**, and the SCD will remain at the peri-microvascular site to be ready to divide asymmetrically again after interaction with host T cells evading from the blood circulation. Black arrowhead in the inset indicates CD8+ remnants of STC from the former CSC asymmetric division. **B.** Thy-1 pericytes (p) accompanying cancer microvasculature exhibit extreme activity in producing Thy-1+ intercellular vesicles (white arrowheads), which collapse into intercellular spikes (arrows) after releasing their content stimulating growth of cancer cells (red asterisks) - compare with moderate pericyte activity in the normal epithelial tissue in Fig. 1C. Red arrowheads indicate proliferation of endothelial cells preparing the vascular extension for the support of an unlimited cancer bulk growth due to the lack of autonomic innervation controlling the pericyte activity in normal tissues. **C.** The CSC asymmetric *in vivo* division repeatedly utilizes circulating CD8+ host T cells to produce cancer "differentiating" cell daughters. The former CSC regresses (rCSC) after producing cancer SCD (cSCD) and cancer "differentiating" cell daughter precursor (cDCDp) with the help of CD8+ host STC. The STC is transferred from rCSC to the cSCD and cDCDp. The postmitotic cancer DCD (cDCD) containing developing nucleus (dN) and regressing STC (rSTC) is formed prior to separation of cDCD from the ongoing new CSC asymmetric division. Arrowheads indicate cSCD extensions into the microvasculature (mv) to attract the host T cells for another cancer ASCD. Adjusted from from (Bukovsky, 2011, 2016: ©Antonin Bukovsky).

contribute to the unlimited grow of cancer cell bulk and the cancer SCDs will divide asymmetrically again after interaction with another host STC (see the yellow arrowhead in the Fig. 5A). The vascular extensions in growing cancers are caused by the lack of vascular autonomic innervations (Ashraf at al, 1996; Terada and Matsunaga, 2001). This is accompanied by extreme activity of vascular pericytes in producing Thy-1+ intercellular vesicles (Fig. 5B - compare with Fig. 1C showing moderate activity of pericytes in the normal squamous epithelium), which stimulate early development of postmitotic cancer DCDs and differentiation of endothelial cell sprouts for vascularization of expanding cancer cell mass.

Fig. 5C shows a detail of CSC asymmetric division in the EOC. Cancer ASCD is a highly complex process consisting of several distinct cell stages that include regressing former CSC, developing a new cancer SCD that exhibits extensions into the adjacent microvasculature to attract a new host CD8+ STC for dividing asymmetrically again, a cancer DCD precursor containing vital host CD8+ STC with unaltered nucleus, and a cancer DCD with developing nucleus and remnants of the regressing CD8+ STC. The observation of the complex events during asymmetric division in EOC indicates a similarity to the events occurring during cellular regeneration in normal tissues. Growth of colorectal cancer exhibits properties that markedly resemble those in the normal intestinal epithelial stem cells (van de Wetering et al., 2002). Although only limited data are available on the modes of asymmetric divisions used by adult mammalian stem cells *in vivo* (Morrison and Kimble, 2006), the main difference is that the regeneration of normal adult tissues is quantitatively controlled by the perivascular autonomic innervation of normal tissues and terminated when the limit is reached (Bukovsky, 2016), but "regeneration" of cancer cells *in vivo* is endless, as perivascular autonomic innervation is absent in both primary (Terada and Matsunaga, 2001) and metastatic (Ashraf at al., 1996) malignant tissues.

From this point of view, the therapeutic effect of immune check point blockades may lie in causing the *in vivo* inability of the perivascular CSCs to divide, due to the lack of assistance of the host's STCs. This causes a marked alteration in the cancer pathobiology due to the lack of postmitotic cancer DCD development and hybridization with the host perivascular pMDCs. Consequently, host perivascular pMDCs may convert into anticancer effector macrophages eliminating the perivascular cancer stem cells. Tumor infiltrating T cells stimulating differentiation of postmitotic cancer DCDs may be converted into cytotoxic effectors causing regression of cancer bulk cells. The disadvantage of checkpoint inhibitors for EOCs therapy is that EOCs have two distinct types of stem cells. The haploid cancer stem cells are not T- cell dependent and can proliferate later, when the checkpoint inhibitors are depleted, since they are preserved in ascites and tumor bulks in the pelvic and abdominal cavities. The same may apply for some malignancies expressing the cancer/testis SCP-1 protein, like malignant gliomas and breast and renal cell cancers (Tureci at al., 1998).

Two distinct cancer stem cell types in pathobiology of EOCs

It is generally believed that a single type of ovarian CSC exists, which is capable of unlimited self-renewal and differentiation. The CSCs represent about 0.01-1% of malignant ovarian cells (Zhang et al., 2008; Burgos-Ojeda et al., 2012; Zhan et al., 2013; Shah and Landen, 2014; Garson and Vanderhyden, 2015). Although a recent article reviewed the *in vivo* role of the ovarian CSC niche (Lupia and Cavallaro, 2017), the specific involvement of the vascular pericytes, pMDCs and CD8+ STCs in the *in vivo* management of perivascular CSCs has not been delineated. Our observations above indicate that the pathobiology of EOCs, and possibly of some other cancer types expressing SCP-1 protein, is more complex *in vivo* and based not on a single uniform ovarian CSC, but on two distinct CSC types, the diploid CSCs undergoing ASCD with the help of the host CD8 T cells at the perivascular regions, and haploid CSCs evolving at the intraperitoneal surface of the cancer cell bulk. During ASCD of the ovarian CSCs, one of the CSC daughters will evolve into the new CSC and the other containing STC will evolve into cancer DCD, like in normal epithelium, but with malignant potential. Cancer DCDs hybridize with perivascular MDCs and join the tumor cell mass, where their "differentiation" and enlargement is stimulated by infiltrating CD8+ T cells. Tumor growth is enhanced by the permanent supply of hybridized postmitotic cancer DCDs. The malignant potential of cancer cells in the tumor exists due to the fact that they lose MDC chromosomes, reverting back to CSCs (Figs. 3, 4A). Additionally, the EOC cells distant from microvasculature exhibit meiosis I cytokinesis resulting in two haploid cancer cells (Fig. 4B), which contribute to the additional genomic instability of the malignant cells. Haploid cancer cells (27 chromosomes) were detected in epithelial lung cancer, that exhibited *in vitro* hyperploidy with 54 chromosomes (Drouin et al., 1993). This shows that haploid EOC cells released from the tumor surface into the abdominal ascites can transform into diploid CSCs and implant to form pelvic and abdominal metastases. Normal ovarian meiosis I events lead to development of two identical haploid cells by symmetric division, with Ki67 expression in both. This contrasts with the ASCD of normal epithelial cells, where SCDs emerge without Ki67. Meiosis I cytokinesis occurs during the development of the new germ cells from the ovarian stem cells, in surface epithelium, in both the adult young and aged human ovaries (Bukovsky, 2015). Ovarian surface epithelial cells are the most common potential source of EOCs, probably due to the genomic instability of haploid germ cells developing during meiosis I in aged women. In young women the haploid germ cells

enter the ovarian cortical veins and are either associated with perivascular granulosa cell nests to form the new primary ovarian follicles, or are quickly eliminated in the ovarian medullary vessels (Bukovsky, 2016). Such events may be absent in older women due to age-related depletion of the immune system reactivity (Mathe, 1997) required for the elimination of superfluous germ cells. The existence of two distinct CSC types in EOCs, and the presence of possibly chemotherapy-resistant haploid CSCs in the abdominal cavity in particular, may contribute to persistence and extreme clinical recurrences of malignancy after primary EOC treatments.

Lessons from early immunotherapy experiments

Immunotherapy dates back to 1868, when the German physicist Busch intentionally infected patients suffering from soft tissue sarcoma with erysipelas. Rapid tumor shrinkage was observed but the response was only partial and tumor recurrence subsequently occurred (Rihova and Stastny, 2015). William B. Coley, the bone sarcoma surgeon, considered to be a "father of immunotherapy", had the idea of stimulating the immune system to reject human cancer cells by injecting streptococcal bacteria into cancer patients. This was met with some success (Richardson et al., 1999; McCarthy, 2006). In the 1970s, a set of experiments involving immunotherapy of cancer provided several important results. Patients with growing cancer had circulating lymphocytes capable of killing *in vitro* their own malignant cells, but this effect was prevented by host circulating antibodies, which blocked effector T cells from killing the malignant cells (Sinkovics et al., 1970; Hellstrom et al., 1971). This serum blocking activity was eliminated by cyclophosphamide (Steele Jr. et al., 1974). A single cyclophosphamide intravenous injection combined with intradermal injection of Corynebacterium parvum toxins resulted in fibrosarcoma regression in 70 percent of the treated mice. Lengthening this treatment caused an increased survival time of the animals (Currie, 1970). The injection of allogeneic spleen cells sensitized *in vitro* caused rejection of tumor allografts (Cohen et al., 1971), suggesting that the stimulation of the immune system alloreactivity was beneficial. Additionally, the BCG vaccine alone had prophylactic and therapeutic effects against the growth of transplanted experimental sarcomas (Simova and Bubenik, 1975). These reports indicated that occasional cyclophosphamide, combined with bacterial toxins and allogeneic sensitization, may be effective for the treatment of human cancers.

Antibody-based therapies, immune checkpoint blockades, cancer vaccines, and chimeric antigen receptor-modified T cells have all shown some preclinical success and have had clinical trials (Chester et al., 2015). Survival of women with EOCs, however, remained poor (Weiderpass and Tyczynski, 2015), despite extensive debulking surgery and high doses of cytostatics. A cure for recurring ovarian cancer continues to be elusive (Coleman et al., 2013). Management of EOCs still mainly consists of cytoreductive surgery and platinum-based chemotherapy. While clinical remissions are obtainable, approximately 70% of patients will relapse and die of disease within 5 years (Baldwin et al., 2012).

Antibody blockade of the T cell molecule CTLA-4 unleashes the body's immune response against malignant tumors. This concept has led to the development of "immune checkpoint therapies" that may prolong the lives of some cancer patients (Littman 2015). Emerging clinical data, however, show limited clinical efficacy of these agents in ovarian cancers, with objective response rates of 10-15% only (Gaillard et al., 2016).

Regression of inoperable EOCs after chemo-immunotherapy without adverse events

Due to the current poor outcomes with conventional treatment of advanced to EOCs and the serious complications of these therapies, former successful cases employing chemo-immunotherapy without adverse complications in two advanced inoperable EOCs is here reviewed. The treatment was based on a series of cancer-related experiments reported in the 1970s (see above).

Review of patient 1 treatment, 61 years old

Before treatment, the patient was in a reactively good physical condition, without evidence of cachexia, and had only a moderate constipation problem. Red and white blood cell counts were normal, but the sedimentation rate was higher (57/90). Explorative laparotomy, performed for painful tumor mass in the left subchondrium in our gynecologic clinic at the Institute for the Care of Mother and Child, Prague, Czechoslovakia, had shown complete infiltration of the pelvis, generalized metastases in the abdominal cavity, and infiltration of the liver with massive metastases of ovarian carcinoma. The surgeon was even unable to perform a palliative colostomy due to the massive convolute of the gut with omentum and stomach and expected that the patient would not survive 14 days. From the abdominal cavity 2.5 liters of the amber color ascites were removed. Histology of ovarian biopsy showed poorly differentiated papillary adenocarcinoma.

After the surgery and a mutual discussion, based on the serious conditions of the case and a review article on experimental studies of cancer treatment published a year ago (Bukovsky, 1975), it was decided to attempt an unusual approach. Instead of common therapy with high doses of cytostatics alone, which were not likely to be effective, it was decided to attempt to induce the anticancer stimulation of the patient's immune system by several methods. Treatments consisted of placental blood-derived IgG, intermittent moderate doses of cyclophosphamide, allosensitization with blood transfusions against alloantigens of cancer cells, and stimulation of the immune system reactivity by

Bacterinum adnexitidicum (BA)/SEVAC (also known as Adnexba) containing toxins from *Enterococci, Escherichia Coli, Neisseria Gonorrhoeae, Staphylococcus Aureus,* and *Streptococci*. Adnexba was formerly used for immunotherapy of acute human female pelvic inflammatory diseases (Kveton, 1972). The 3.8 ml of IgG, commercially separated from the retroplacental blood and expected to contain T cell unblocking antibodies (Hellstrom at al., 1971), was injected intramuscularly the first day after surgery, followed the next day by 1.9 ml and intravenous injection of 400 mg cyclophosphamide. Three days and ten days after surgery the patient received 500 ml blood transfusions to stimulate immune system alloreactivity, and high titers of Adnexba were injected intradermally two to three times a week. Two weeks after explorative laparotomy the patient was released to come once a week for intradermal injections of Adnexba. Two months after explorative laparotomy the oral use of 50 mg cyclophosphamide tablet each day for a period of four weeks, combined with weekly intradermal injections of Adnexba toxins, was begun. Ten weeks after explorative laparotomy a fist size tumor was still detected in the left subchondrium but no ascites. After finishing the cyclophosphamide treatment, it was decided to discontinue all anticancer therapy.

By two weeks after discontinuation of all anticancer therapy a relatively prompt tumor regression in the left subchondrium had occurred. Six months after surgery a normal liver size was noted, and irrigoscopy demonstrated normal conditions of the colon. A "second look" surgery was performed. During re-laparotomy the surgeon found the gut convolutions movable and entirely free of adhesions and the peritoneum smooth. The pelvic cavity was blocked by coalescent remnants of the cancer process, exhibiting blue/brownish transparent cystic formations. The liver had regenerated into a normal healthy condition, without palpable metastases. The rectum exhibited several wrinkled nodes. A hysterectomy with salpingo-oophorectomy was performed. At this time the ovarian histology showed shrinking residual malignant adenocarcinoma with vigorous foci of degenerating malignant tissue. No evidence of fresh malignant growth was found. One week after the second look surgery the patient received a single blood transfusion and was considered to be cured. Five months after surgery the patient underwent a balneotherapy in warm mineral springs. A month later manifestation of constipation and signs of cancer recurrence in the abdominal cavity recurred. Treatment with the cyclophosphamide tablets alone was without results, and the patient soon died of a massive pulmonary embolization. An autopsy showed a marked recurrence of malignancy in the abdominal cavity (Bukovsky and Presl, 1986). The course of the patient's hospital cancer treatment is summarized in Table 1.

Even though regression of advanced cancer was attained, we were unable to prevent its relapse. This led to skepticism about the value of the immunotherapeutic approach, but eventually thoughts turned to analyzing what had caused the cancer regression and what possible errors resulted in its recurrence. It was speculated that one of the possible causes of relapse was the second look surgery with hysterectomy and salpingo-oophorectomy while there was ongoing cancer regression. Possibly, the patient needed more than only subsequent blood transfusions. The recurrence was also treated only with cyclophosphamide without Adnexba toxins. The warm spring balneotherapy might have also significantly contributed to the initiation of the EOC relapse.

Review of patient 2 treatment, 63 years old

A 63-year-old woman presented with marked ascites. A high sedimentation rate (73/139) was detected. On August 15, an explorative laparotomy was performed for excessive ascites and a distinctive retrovaginal mass on examination. A malignant ovarian mass involving a large amount of the abdominal cavity was discovered, and 6 liters of ascetic fluid was removed. Histology

Table 1. Survey of patient 1 inoperable EOC treatment.

Days since exploratory laparotomy	Case treatment course
0	Explor. laparot.: 2.5 L of ascites drained
1	IgG 3.8 ml i.m,
2	IgG 1.9 ml i.m, CF 400 mg i.v.
3	1st BT 500 ml; BA i.d. high titers, 3 times a week
13	2nd BT 500 ml, BA i.d. 2 times a week
14	Released from hospital. Attended once a week for BA i.d. application
60	CF 50 mg tablets daily for 28 days and BA i.d. once a week.
88-183	No therapy period
102	Sudden regression of abdominal tumors 14 days since beginning of no therapy period
184	Irrigoscopy: regular findings
188	Second look laparotomy with hysterectomy & adnexectomy
196	BT 500 ml

IgG, Retroplacental immunoglobulin G; BT, blood transfusion; BA, Bacterinum adnexitidicum; CF, cyclophosphamide.

showed a poorly differentiated solid ovarian carcinoma. 3.8 ml of retroplacental IgG was injected intramuscularly the first day after surgery, followed the next day by 1.9 ml. Two weeks after laparotomy cyclophosphamide 400 mg was injected intravenously. Two days later weekly intradermal allosensitization using 0.5 ml of lympho-leukocytic concentrate (allogeneic blood buffy coat) mixed with the high titers of Adnexba toxins was initiated. After the first allosensitization combined with Adnexba toxins, the patient had a common strong reaction with fever, but subsequent applications were without any side effects. Three weeks after laparotomy, another 6 liters of ascetic fluid were drained. The first blood transfusion was performed two weeks later. At this time ascites were again present, but fewer than before. No drainage was performed. A second blood transfusion of 250 ml was performed 10 days later, and from the remaining 250 ml of blood the white blood cell buffy coat was collected for the continuation of allosensitization. The patient's blood counts remained in normal ranges, and the sedimentation rate was significantly lower (30/57) compared to before treatment. Weekly therapy with high doses of Adnexba toxins continued after release from the hospital. Two and half months after laparotomy, a small amount of ascites was still present and 0.5 ml of allogeneic buffy coat was injected intradermally. At this time daily cyclophosphamide tablets were set at 50 mg for 4 weeks, but without weekly injections of Adnexba toxins. Blood smears showed the marked depletion of lymphocytes. Subsequently the ascites increased, and 8 liters of yellowish ascetic fluid were drained. Ten days later three more liters were removed. 0.5 ml of allogeneic buffy coat was injected intradermally. In the next ten days ascites remained unchanged, and 0.5 ml of allogeneic buffy coat mixed with Adnexba toxins was injected intradermally to both groins. One month later, without any further therapy, the abdomen was soft, ascites undetectable, and no abdominal or pelvic mass was palpable. The previous month's exact treatment was repeated. Further information on this patient is not available (Bukovsky and Presl, 1986). The patient's course of hospital cancer treatment is summarized in Table 2.

Comparisons of the reported inoperable advanced EOCs treatments

The reported chemo-immunotherapy of inoperable EOCs was not accompanied by any adverse events. It has been reported that cyclophosphamide, in addition to its cytotoxic effect on cancer cells and leukocytes, improves the effectiveness of immunotherapy by immunomodulatory effects. Mechanisms include suppression of Treg cells, induction of homeostatic proliferation of T and B lymphocytes, facilitation of cancer infiltration by lymphocytes, and the emergence of a cytokine storm during recovery due to lymphocyte homeostatic proliferation (Zicchedu et al., 2013). Therefore, intermittent moderate cyclophosphamide treatment is an important component of effective immunotherapy. During cancer treatment, blood transfusions stimulate immune system reactivity against the alloantigens expressed by cancer cells (Bukovsky and Presl, 1985), and provide unaltered circulating white blood cells, which may also contribute to anticancer immunity.

It appears that the treatment of the first patient with earlier applications of cyclophosphamide, blood transfusions and Adnexba toxins after exploratory laparotomy (Bukovsky and Presl, 1986) was more successful, since it prevented the continuing development of ascites. Treatment with cyclophosphamide tablets alone without Adnexba toxins in

Table 2. Survey of patient and cancer treatment.

Days since exploratory laparotomy	Case treatment course
0	Explor. laparot.: 6 L of ascites drained
1	IgG 3.8 ml i.m.
2	IgG 1.9 ml i.m.
14	CF 400 mg i.v.
16	Allogeneic Buffy Coat (ABC) 0.5 ml mixed with high titer BA i.d.
25	6 L of new ascites drained
39	Blood transfusion (BT) 500 ml
57	Patient released for 10 days
67	Admitted continuing Immunotherapy
70	BT 250 ml (remaining 250 ml of blood used for preparation of 0.5 ml ABC doses)
72	BA i.d. weekly
106	CF 50 mg tabl. daily for 4 weeks without BA treatment
142	End of CF treatment
147	8 L of ascites drained
157	3 L of ascites drained. ABC, 0.5 ml i.d.
164-192	No therapy period
164	Small ascites only. ABC+BA i.d. to both groins
192	Soft abdomen, no tumor or ascites palpable. Vaginal Examination: no palpable pelvic tumor

the second patient was not effective. Nevertheless, the later intradermal injection of 0.5 ml of allogeneic buffy coat mixed with Adnexba toxins to both groins without any additional treatment for one month thereafter, caused regression of abdominal and pelvic cavity tumor masses. The temporary use of bacterial toxins can prevent cancer progression, but they are not capable of causing ovarian cancer regression, such as that seen during the treatment-free period after the combination of Adnexba toxins with the cyclophosphamide or with allogeneic buffy coat. If not available, the Adnexba toxins can be replaced by BCG vaccine exhibiting *in vivo* the prophylactic and therapeutic effects on mammalian cancer growth (Simova and Bubenik, 1975).

The data reported originate from the former and recently discovered commentary not referenced in the PubMed on the detailed description of the clinical courses of patient 1 treatment (Bukovsky and Presl, 1986), not available previously (Bukovsky, 2016), and patient 2 treatment (Bukovsky and Presl, 1986) also reviewed here. The data suggest that sequential immunotherapy with cyclophosphamide eliminated antibodies blocking anticancer T cell effectors. Blood transfusions stimulated immune reactivity against cancer alloantigens, and bacterial toxins were enhancing anticancer immune reactivity. These should be considered immediately after exploratory laparotomy (see Table 1). Intradermal injections of bacterial toxins, mixed with allogeneic buffy coats, should be given bilaterally close to lymph nodes draining the area with the primary cancer, i.e., the groin skin in EOCs. The described chemo-immunotherapy should incorporate several weeks of a treatment-free interval, when immunotherapy stops the cancer progression and the modulated immune system with enhanced anticancer reactivity is capable of rejecting primary cancer cells and their metastases, and also participare in the regeneration of tissues affected by cancer metastases into normal healthy conditions.

The patients with advanced (inoperable) EOCs considered for these treatments should be in a relatively good condition without cachexia and with normal values of the red and white blood cells. This treatment might also be applicable for other epithelial cell cancers, e.g. advanced colorectal cancers, and possibly for some nonepithelial malignancies. What remains unresolved is an effective avoidance of frequently recurring EOCs, since the prevention of EOC relapses has been unavailable, and remains problematic even at the present time. Continuous stimulation of the patients' own immune system anticancer reactivity, and reactivity against cancer stem cells in particular, the goal of this proposed approach, could improve outcomes.

Long lasting cancer survival depends on effective prevention of cancer relapses

One medication that appears to have potential to decrease the risk of cancer and improve cancer survival is oral metformin (Evans et al., 2005). Metformin use has a protective effect for recurrences of ovarian and endometrial cancers (Febbraro et al., 2014). It inhibits ovarian CSCs (Zhang et al., 2015) and selectively kills CSCs that resist chemotherapy and cause cancer relapses (Hirsch et al., 2009).

An additional option is the consumption of raw shiitake mushrooms (Bomford and Moreno, 1977; Kidd, 2000; Hazama et al., 2009; Avinash et al., 2016). They stimulate immune system mediated regression of cancer cells (Vetvicka and Vetvickova, 2014). The active anticancer component of shiitake is the thermolabile beta-glucan lentinan (Dai et al., 2015), which causes toxic shiitake dermatitis (Mendonca et al., 2015). To prevent shiitake dermatitis, shiitake mushrooms are recommended to be consumed after prolonged cooking (Boels et al., 2014), which, however, degrades the thermolabile lentinan. Lentinan exhibits marked anticancer and antimetastatic effects in numerous tumor/host systems, and inhibits development of chemical and viral carcinogenesis (Suzuki et al., 1994). It also stimulates macrophage cytotoxicity against metastatic cancer cells (Ladanyi et al., 1993), and increases survival of cancer patients when combined with chemotherapy (Ina et al., 2013). Recurrent metastatic ovarian cancer regressed without chemotherapy, when the immunotherapy was followed by three applications of 2 mg lentinan every two weeks (Fujimoto et al., 2006).

Besides that, propolis ethanol extract was reported to strengthen the body's immune system and activate the thymus, exhibit anti-infection and anticancer effects, significantly decrease systolic blood pressure, and reduce blood glucose levels in diabetes, and causeds significant decrease of systolic blood pressure in spontaneously hypertensive rats, but had no effect on normal rat controls (Kubota et al., 2004). Propolis ethanol extract was reported to attenuate blood glucose and plasma cholesterol in ob/ob mice (Kitamura et al., 2013). Ethanolic soluble derivative of propolis extract administered to diabetic mouse models increased the number of immunoregulatory T cells, causing decrease of blood sugar levels (Rifa'i and Widodo, 2014). Propolis administered to rats by oral gavage reduced blood glucose levels in diabetes and might be beneficial for the treatment of periodontitis (Aral et al., 2015). Propolis flavinoids liposome was able to effectively activate the cellular and humoral reactivity of immune cells in mice, including proliferation rates of splenic lymphocytes (Tao et al., 2014). Animal and cell culture experiments indicated that propolis utilization strengthens the body's immune system and activates the thymus (Sforcin, 2007; Missima and Sforcin, 2008; Pagliarone et al., 2009; Fan et al., 2014). In addition to its anticancer effects, immune system stimulation by local and/or systemic use of honey bee propolis also regenerated lost hair and stopped teeth loss, regressed varicose veins, improved altered hearing, and lowered high blood pressure and sugar levels. For propolis

tincture preparation on the systemic use and local effects for the lost hair regeneration, regression of varicose veins, and dental preservation see Fig. 17 in Bukovsky (2016), and accompanying text.

An otherwise untreated rectal cancer was resolved after a severe dermatitis caused by ingestion of uncooked raw shiitake mushrooms. Subsequent weekly consumption of a single larger raw shiitake mushroom in salads prevented any malignant recurrence over the next 29 years, without subsequent episodes of dermatitis (Bukovsky, 2016). This was soon accompanied by 1 gr Meltformin ER twice daily for the treatment of type II diabetes. The emergence of the skin dermatitis after consumption of the raw shiitake mushroom resembles the emergence of erysipelas after injection of Coley toxins, which have caused regression of some epithelial cancers and soft tissue sarcomas (Richardson at al., 1999).

These observations show that chemo-immunotherapy with cyclophosphamide causing depletion of endogenous antibodies blocking anticancer T cell effectors, accompanied by sensitization of the immune system against alloantigens of cancer cells by blood transfusions and or intradermal injections of allogeneic buffy coats, and enhancement of immune system reactivity by bacterial toxins can be augmented with daily oral doses of metformin and weekly consumption of the raw shiitake mushroom. This should enable cancer regression and regeneration of the metastatically altered tissues, without need of an extensive debulking cytoreductive surgery or treatment with high doses of cytostatics in advanced EOCs, and possibly some other types of cancers. Continuing daily metformin and weekly raw shiitake mushroom consumption after cancer regression can ensure a long lasting prevention of cancer recurrences, without emerging dermatitis and/or pruritus. The same may apply for the prevention of cancer relapses after their regression induced by the standard cytoreductive surgery and high dose chemotherapy.

Discussion

Current cancer treatments almost entirely consist of continuous utilization of anti-cancer drugs or immunotherapies. Available data indicate that current cancer immunotherapy by immune checkpoint blockades may result in high grade multiorgan alterations, and even in patient death (Bertrand at al., 2015). The causes of the immune adverse events lie in alteration of the physiological immune system's role in tissue homeostasis. Cancer treatments with high doses chemotherapy also cause adverse events that alter the quality of patient' lives. During the last several decades the treatment of advanced EOCs by various approaches has not substantially improved the extended survival of patients. Reviewed observations indicate that chemo-immunotherapy consisting of intermediate moderate cyclophosphamide doses causing depletion of endogenous antibodies blocking T cell anticancer effectors (Steele Jr. et al., 1974; Le and Jaffee, 2012), sensitization of the immune system against alloantigens of cancer cells by blood transfusions and/or allogeneic blood buffy coats, and enhancement of immune system reactivity by weekly intradermal bacterial toxins can be augmented with daily doses of 2 gr Metformin ER and weekly consumption of the larger raw shiitake mushroom, or 2 mg lentinan. This should enable faster cancer regression accompanied by regeneration of the metastatically altered tissues, without a need of multivisceral cytoreductive surgery, which can have life-threatening consequences (Milek et al., 2016), or accelerate treatment with high doses of cytostatics in advanced EOCs, and possibly other cancer types. The continuing daily metformin and weekly larger raw shiitake mushroom or purified lentinan consumption after cancer regression can be followed by a long-lasting prevention of cancer recurrences, without recurring dermatitis and/or pruritus. The same may apply for the prevention of cancer relapses after regression induced by standard cytoreductive surgery and high dose chemotherapy.

Beside that, cancer regression may be induced by consumption of the larger raw shiitake mushroom alone, if it is followed by severe toxic dermatitis lasting over one week. The weekly consumption of the larger raw shiitake mushroom alone, or a daily smaller portion of it preserved in a freezer, may also prevent cancer evolution, such as an appearance of lung cancer, which often develops in the heavy tobacco smokers.

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