Radiotherapy and Oncology xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Radiotherapy and Oncology

journal homepage: www.thegreenjournal.com

Potential of memory T cells in bridging preoperative chemoradiation and immunotherapy in rectal cancer

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ARTICLE INFO

Article history:
Received 23 October 2017
Received in revised form 20 March 2018
Accepted 2 April 2018
Available online xxxx

Keywords:
Rectal cancer
Memory T cells
IL-2
Metformin
mTOR

ABSTRACT

The management of locally advanced rectal cancer has passed a long way of developments, where total mesorectal excision and preoperative radiotherapy are crucial to secure clinical outcome. These and other aspects of multidisciplinary strategies are in-depth summarized in the literature, while our mini-review pursues a different goal. From an ethical and medical standpoint, we witness a delayed implementation of novel therapies given the cost/time consuming process of organizing randomized trials that would bridge an already excellent local control in cT3-4 node-positive disease with long-term survival. This unfortunate separation of clinical research and medical care provides a strong motivation to repurpose known pharmaceuticals that suit for treatment intensification with a focus on distant control. In the framework of on-going phase II-III IG/IMRT-SIB trials, we came across an intriguing translational observation that the ratio of circulating (protumor) myeloid-derived suppressor cells to (antitumor) central memory CD8+ T cells is drastically increased, a possible mechanism of tumor immuno-escape and spread. This finding prompts that restoring the CD45RO memory T-cell pool could be a part of integrated adjuvant interventions. Therefore, the immunocorrective potentials of modified IL-2 and the anti-diabetic drug metformin are thoroughly discussed in the context of tumor immunobiology, mTOR pathways and revised Warburg effect.

Current standard treatment for locally advanced rectal cancer is radiotherapy with 5-fluorouracil (5-FU) or oral capecitabine, followed by total mesorectal excision (TME). This regimen improves the local control with a local recurrence rate about 5% [1], but without significantly improving the long-term survival rate. The distal recurrence rate remains around 30% [2], representing the main cause of death in rectal cancer [3]. For this reason, oxaliplatin and targeted therapies, such as bevacizumab and cetuximab were evaluated in the neoadjuvant setting but with conflicting results (partially covered by our Section 2) [4–11]. To achieve risk-adapted and less toxic treatments, the approaches of omission radical surgery or radiotherapy, or intensity-modulated radiotherapy without chemotherapy are under investigation in selected subgroups of patients [12–15]. The success of immune checkpoint blockades in the treatment of advanced melanoma and lung cancer patients revolutionized oncology [16,17]. Recently, in colorectal cancer (CRC), the anti-PD-1 drug pembrolizumab was approved to treat metastatic/refractory microsatellite instability-high (MSI-H) patients [18]. Of note, MSI-H exists in about 15% of CRC [19], indicating that besides immune checkpoint blockades, other immune boosting approaches should be explored. Immunological memory is a fundamental feature of adaptive immunity. A higher density of memory T cells in CRC is a favorable prognostic factor for overall survival [20]; in contrast to the 'protumor' inflammatory markers at systemic level, such as neutrophil-to-lymphocyte ratio (NLR) and myeloid-derived suppressor cells (MDSC) (in-depth overviewed in our Section 3) [21,22]. With the increased understanding of the mechanisms that govern the formation of memory T cells, their ability to acquire longevity, and self-renewal, it becomes conceivable to adopt memory T cells to provide enduring anti-tumor effects.

Metformin, an anti-diabetic biguanidine, is probably the most exciting pharmaceutical in the pipeline of drug repurposing with over 100 clinical trials in oncology. While its antitumor properties are detailed elsewhere [23], we acknowledge here the intriguing fact that metformin as a mammalian target of rapamycin (mTOR) inhibitor might restate the pool of pluripotent CD45RO memory

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https://doi.org/10.1016/j.radonc.2018.04.003
0167-8140/

T cells. Of note, these immunocorrective effects are beyond the already identified immune checkpoints (as PD-1/PDL-1) that preferentially operate in more differentiated effector T cells within the tumor site [16,17]. Accumulating evidence suggests that effector T cells resemble tumor cells characterized by the Warburg metabolism and regulated by mTOR pathways to sustain proliferation [24,25]. In contrast, memory T cells rely more on fatty acid oxidation regulated via AMP-activated protein kinase (AMPK) signaling pathways [24,26]. mTOR inhibitors or AMPK activators including metformin therefore have a potential to initiate the effector to memory T cell transition [26,27]. Besides a metabolic switch, memory T cells require a second trigger to maintain their longevity/expansion, which is largely controlled through the CD122 chain (Rβ) of the IL-2 receptor [28]. Opposed to that, CD25 chain (Rα) signaling is responsible for the outgrowth of Tregs, a physiological mechanism to inhibit and shutdown T-cell stimulation [29]. Therefore, section 4 describes the state-of-the-art tools of molecular immunology, which offer an elegant solution to restrain (protumor) CD4 regulatory T cells (Tregs) in favor of (antitumor) memory CD8 T cells by using a CD122-biased IL-2. Our understanding is that an efficient re-instatement of T-cell memory at systemic level (blood and lymph nodes) could be obtained by the two key triggers: (1) graded mTOR inhibition by metformin and (2) optimal cytokine stimulation by a CD122-biased IL-2.

We believe that our review will encourage both researchers and doctors to (re)consider metformin for immunological evaluations with the following take-home messages: (1) mTOR inhibitors appear to favor T-cell memory and offer immunocorrection at systemic level, in contrast to PD-1/PD-L1 checkpoint inhibitors that operate in the tumor; (2) metformin, an anti-diabetic drug and mTOR inhibitor, is already repurposed for targeting tumor metabolism in ongoing clinical trials, yet needs a next round of repurposing for long-term immunocorrective interventions and (3) CD122-biased IL-2, preferentially expanding the memory T cells, may be incorporated with metformin to sustain the adaptive immune response.

Preoperative chemoradiotherapy in rectal cancer

The management of CRC, and particularly locally advanced rectal cancer, has historically established new standards of clinical research and medical care that illustrated the importance of (i) a multidisciplinary approach in treatment modalities, (ii) collaborative efforts in organizing international large-scale randomized trials, and (iii) a strong dedication of teams across the world to examine alternative interventions based on technical and pharmacological developments. Despite standing just at its beginning, the 21st century has already introduced into practice two major paradigms – the TME and preoperative chemoradiation, which together secure the loco-regional control in rectal cancer above 90%. While the procedure of TME is globally accepted as the only golden standard of radical surgery [30], the role of chemoradiation continues to broaden and evolve leaving enough room for pre- versus post-operative regimes, and radiation or chemotherapy alone versus their concomitant application [12–15]. As a result of successful German, Dutch, French, Polish and other trials, the European schools put forward preoperative 5-FU/capecitabine-based chemoradiation, which markedly decreases local tumor recurrence and seems to minimize the risk of patient under-treatment and hence the necessity to rely on further aggressive (and more toxic) adjuvant options [31–36].

Another paradigm shift may be referred to our growing understanding that the clinical stage of locally advanced cT3-4 node-positive rectal cancer represents, in fact, heterogeneous diseases with variable clinical outcomes [12,15,37]. Therefore, the optimization of personalized treatment plans may benefit from a patient-tailored separation of chemo- and radiotherapy, a recent and unexpected turn in the view of modern combined strategies that have guided treatment intensification for decades. As an example, the team of Schrag D et al. opted in their PROSPECT trials for intensified chemotherapy FOLFOX and selective radiation for non-responders only [38–40], while Valentini V et al. have chosen more radiation up to 54 Gy using high-precision IMRT-SIB, intensity-modulated radiotherapy with simultaneous integrated boost [41–43]. Those diverged programs, however, pursue the same twofold goal – to lower delayed toxicity/morbidity despite an increased tumor cytoreduction and to improve distant control in high-risk patients by restraining metastatic spread, the main cause of cancer-related deaths [14]. On the other hand, low-risk patients staged T3N0M0 with an upper rectal location might favor from an omitted over-treatment, linked to neoadjuvant chemoradiation [44,45], once the diagnostics of involved CRM (circumferential resection margin) and lymph nodes by MRI is improved [13,45]. CRM remains to be a critical objective parameter for treatment planning, and its narrow margin (less than 1–2 mm) next to a low tumor location and extended vascular, lymphatic and perineural invasion indicates an increased risk of local recurrence and compromised prognosis [46,47]. Yet, even a low-risk tumor may be understaged due to the limitations of CT/MRI scanning to address the micro-disease, a not infrequent situation discovered by postsurgical pathology that requires adjuvant interventions. This fine-tuning of disease-oriented chemoradiation, however, proceeds by slow and incremental steps since a differential analysis of risk groups (low versus intermediate versus high) would require a big cohort of randomized patients given the already excellent level of local control in the TME era. Therefore, overall survival rates as the primary end-point are hardly feasible, and many on-going phase II trials contain inherent shortcomings by re-focusing on non-inferiority, pCR by Dworak and short-term disease-free survival, including our own studies [48,49].

To improve distant control and overall survival rate, a number of intensified strategies based on oxaliplatin, targeted and biological agents have been recently explored. According to the results from the ACCORD 12, STAR-01, PETACC-6 and NSAPB R-04 randomized trials, the addition of oxaliplatin increased toxicity, but failed to improve the early and long-term endpoints, such as the pCR, disease-free survival and overall survival [4–7]. Conversely, in the phase III CAO/ARO/AIO-04 trial the addition of oxaliplatin was well tolerated, associated with increased pCR rate and disease-free survival [8,9]. In addition, preliminary results from the large multicenter FORWARC study demonstrated that the pCR rate was significantly higher in the arm combining mFOLFOX6 with radiotherapy compared to the arm of 5-FU with radiotherapy [8,9]. Among biological agents representing monoclonal antibodies, the EGFR blocker cetuximab showed disappointing low rates of pCR [10]. The VEGF blocker bevacizumab demonstrated a trend toward improved clinical outcomes but at the cost of increased surgical complications [11]. Altogether, significant advancements in the management of locally advanced rectal cancer have occurred over the last decades, resulting in improved local control rates. However, the risk of distant metastases remains an ongoing problem and the major obstacle to improve the survival rate, requiring novel strategies [50].

Immunobiology of colorectal cancer

Immunoprofiling of colorectal cancer at local and systemic levels

Over the last decade, inflammatory and immune biomarkers underwent extensive investigation in many tumor types, and CRC

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is one of the most studied in the context of prognostic significance. In contrast to other malignancies, macrophages and Tregs are not qualified as risk factors suggesting an alternative polarization or distinct functions along chronic inflammation, the key event in colon carcinogenesis [51,52]. Next, CRC is associated with expanded granulocytic immunosuppressive networks, resembling renal cancer but not melanoma in that aspect, where circulating MDSCs are of monocytic origin [22,53]. At the local level, an in depth analysis of tumor infiltrating immune cells revealed that both $CD3^+$ and $CD8^+$ T lymphocytes significantly correlated with disease-free and overall survival, a basis for the prognostic immunoscore system [54,55]. In addition, $CD45RO^+$ memory T cells appeared to be a strong indicator of improved clinical outcome with evidence emerging from varying layers [20]. By immunohistochemical staining, increased memory $CD45RO^+$ T cells at the primary site were associated with a low incidence of tumor recurrence [54], the absence of signs of early metastatic invasion and increased overall survival [20]. At metastatic sites (liver and lung), it was an independent prognostic factor for overall survival [56]. These findings have been summarized in Table 1 [20,54,56–69] and a recent meta-analysis [70]. Given that in situ memory T cells predict long-term oncological outcome, it is plausible that memory T cells migrate to distant sites and provide enduring anti-tumor effects due to their trafficking and self-renewal characteristics.

At a systemic level, the Glasgow prognostic score (GPS), referred to as an elevated level of C-reactive protein and hypoalbuminemia in plasma, is associated with poor cancer-specific survival independently from TNM in stages II-III CRC [71,72]. An increase in the NLR in blood was demonstrated to predict poor outcomes in CRC patients following the resection of the primary tumor or liver metastases [21,73,74]. This could be explained by the fact that local T-cell infiltration is associated with tumor immunosurveillance, while systemic inflammation correlates with immunosuppression and poor outcome. Therefore, the activation of (potentially) anti-tumor T-cell responses and/or disruption of a tumor supporting immunosuppressive network appear to be an appealing strategy to improve long-term survival in CRC. Unfortunately, so far various immunostimulatory strategies fail to increase the overall survival rates in CRC. For immune checkpoint blockade, only the subgroup of tumors with microsatellite instability currently seems to be a suitable candidate due to the increased load of (immunogenic) frameshift and missense mutations [75,76]. This observation is in line with the success of immunotherapy in melanoma, renal cell carcinoma and non-small lung cancer, which are all marked by high mutational burden. Of note, even for the immunogenic tumors, only a small portion of patients experiences clinical benefit of immune checkpoint blockade. Therefore, identification and validation of reliable biomarkers that drive the activity of immunotherapeutic agents are under intensive investigation with a series of innovative candidates, such as mutational load and immune cell populations [77]. Interestingly, baseline NLR is reported to be significantly associated with the outcome of ipilimumab-treated melanoma patients [78], indicating its potential to be explored as a predictive biomarker for checkpoint blockade. Altogether, the immune paradox in CRC is that the immunoscore based on tumor T-cell infiltration represents a strong prognostic parameter in addition to TNM yet does not predict the outcome of immunotherapy, possibly because its potential is confined by immunosuppressive networks fostered by inflammation.

Multiple reasons may contribute to an apparent conflict between the prognostic and predictive parameters in cross-talking immune compartments, e.g. granulocytic MDSC and T cells. Our analysis of MDSC in preclinical CRC models and in rectal cancer patients indicated that overexpressed arginase-1 (Arg) in granulocytes may lead to L-arginine depletion and thereby to dual protumor effects that involve both T-cell suppression and functional inactivation of M1 macrophages, ultimately causing tumor cell radioprotection through the arrest of nitric oxide synthesis [79]. Moreover, the nature of inflammation in the tumor microenvironment may also impact the response of a tumor to immunotherapy. Acute inflammation is known to activate cytotoxic $CD8^+$ T cells, a terminally differentiated and short-living subset, whereas chronic inflammation induces the functional exhaustion of $CD8^+$ T cells due to a growing deficiency of the long-living memory pool [80]. This could explain the elevated levels of NLR, an established inflammatory score, which has been repeatedly demonstrated to correlate with poorer survival in CRC [21,73,74]. Indeed, the increase in NLR coincides with a drastic outgrowth of inflammatory Arg^+ granulocytes in the circulation, which may provoke a dysregulated infiltration of the tumor by Arg^+ MDSC over T cells [79]. In our preliminary data set (Fig. 1), a 1.7-fold increase of median NLR was observed in rectal cancer patients as compared with donors (panel 1). In addition, an escalating increase could be detected in the highest quartile of NLR values (dotted line) in the cumulative curves (panel 2), a rationale for a widely used prognostic cut-off of 5.0 [21]. As a result, the levels of (protumor) Arg^+ neutrophils and MDSC were increased by 3.9 and 5.7-times

Table 1
Main characteristics of studies investigating the prognostic value of CD45RO memory T cells in colorectal cancer. Abbreviations: CSS = cancer specific survival; DFS = disease-free survival; NR = not reported; OS = overall survival; PFS = progression-free survival.

Ref	Authors (year)	Rectal/colon	No. Patients	Diseases stage	Cut off point	Counting site	Significant outcomes
[20]	Pages et al. (2005)	287/672	959	Duke's A-D	High: 250 cell per square mm	Tumor	OS, DFS
[54]	Galon et al. (2006)	245/162	415	I–III	Median	Tumor center and invasive margin	OS, DFS
[57]	Salama et al. (2009)	NR	967	II–III	Median	Invasive margin	OS
[58]	Pages et al. (2009)	NR	411	I–II	Minimum *p* value	Tumor center and invasive margin	OS, DFS
[59]	Peng et al. (2010)	0/72	72	IIIB	High: ≥24 cells per high-power field	Tumor	OS
[60]	Lee et al. (2010)	0/53	53	II	Mean	Tumor center and stroma	OS,DFS
[61]	Nosho et al. (2010)	153/615	738	I–IV	First to fourth quartile	Tumor center and stroma	OS, CSS
[62]	Zlobec et al. (2010)	NR	920	NR	NR	tumor	CSS
[63]	Chew et al. (2011)	NR	120	I–IV	Median	Tumor	CSS
[64]	Formica et al. (2013)	5/26	31	Grade 1–3	Median	Blood	PFS
[56]	Lee et al. (2013)	0/79	79	IV	Mean	Tumor center and metastasis	OS
[65]	Koelzer et al. (2014)	30/99	130	I–IV	Mean	Tumor center and stroma	OS
[66]	Brunner et al. (2014)	82/119	201	IV	Median	Tumor center and stroma	OS
[67]	Kim et al. (2015)	258/539	797	I–IV	Median	Tumor center and invasive margin	OS, PFS
[68]	Wang et al. (2015)	185/0	185	I–III	Median	Tumor	DFS
[69]	Chen et al. (2016)	148/152	300	I–IV	x-tile software	Tumor	OS, DFS

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respectively (panels 3–4). This raise is opposed to a 1.8-fold drop in (antitumor) $CD8^+$ T-cell numbers and more importantly at the cost of a 1.7 to 7-fold decline of memory T cells with the highest impact on the central memory subset (panel 5–6). Extrapolating from those data, a 2-fold increase in NLR may culminate in a more than 10-fold burst of MDSC over memory T cells, thus raising the concern of whether these immune arms are instrumental in compromising both the adaptive immunity and curability in relapsed patients. Further decoding of NLR in terms of distinct functional subsets within the neutrophil and lymphocyte compartments is required to project accumulating translational findings into future immunocorrective strategies. Besides, the genetic signature of tumor cells including microsatellite instability, methylation and mutation status emerges as an essential orchestrating mechanism that pre-shapes the nature of tumor immune surveillance and escape [81,82].

Warburg effect and re-instatement of T-cell memory

The current developments in tumor-promoting MDSC have been extensively discussed elsewhere [83]. Here we primarily reflect on potentially antitumor memory T cells whose functionality can be apparently reprogrammed through the mTOR pathway. Three decades ago, the role of helper-inducer T cells was reinterpreted using antibodies against different isoforms of CD45R, where $CD45RO^+$ T cells have emerged as a memory subset opposed to naïve $CD45RA^+$ T cells [84]. In parallel, the multi-protein complex TOR was characterized by Heitman J et al. in yeast as a gateway to cell growth and proliferation, and mTOR was next identified by converging efforts of several teams [85]. After the seminal work of Sallusto F et al., memory T cells can be further divided into central memory and effector memory subsets using CCR7 and CD62L, a chemokine receptor and a selectin respectively, which control homing to secondary lymphoid organs [86]. However, the memory T-cell pool in tissues is still recognized at a glance by staining CD45, a transmembrane tyrosine phosphatase that switches the isoform RA to RO upon alternative splicing [20]. This particular activation switch was crucial in comprehending a selective loss of functional memory T cells in immunodeficiency (e.g. HIV). It is noteworthy to remind that the role of CD45RO memory T cells has been recently revived in the domain of chronic viral infections and immunosenescence and their metabolism is now under dissection across the mTOR pathways tightly linked to the Warburg effect [87,88].

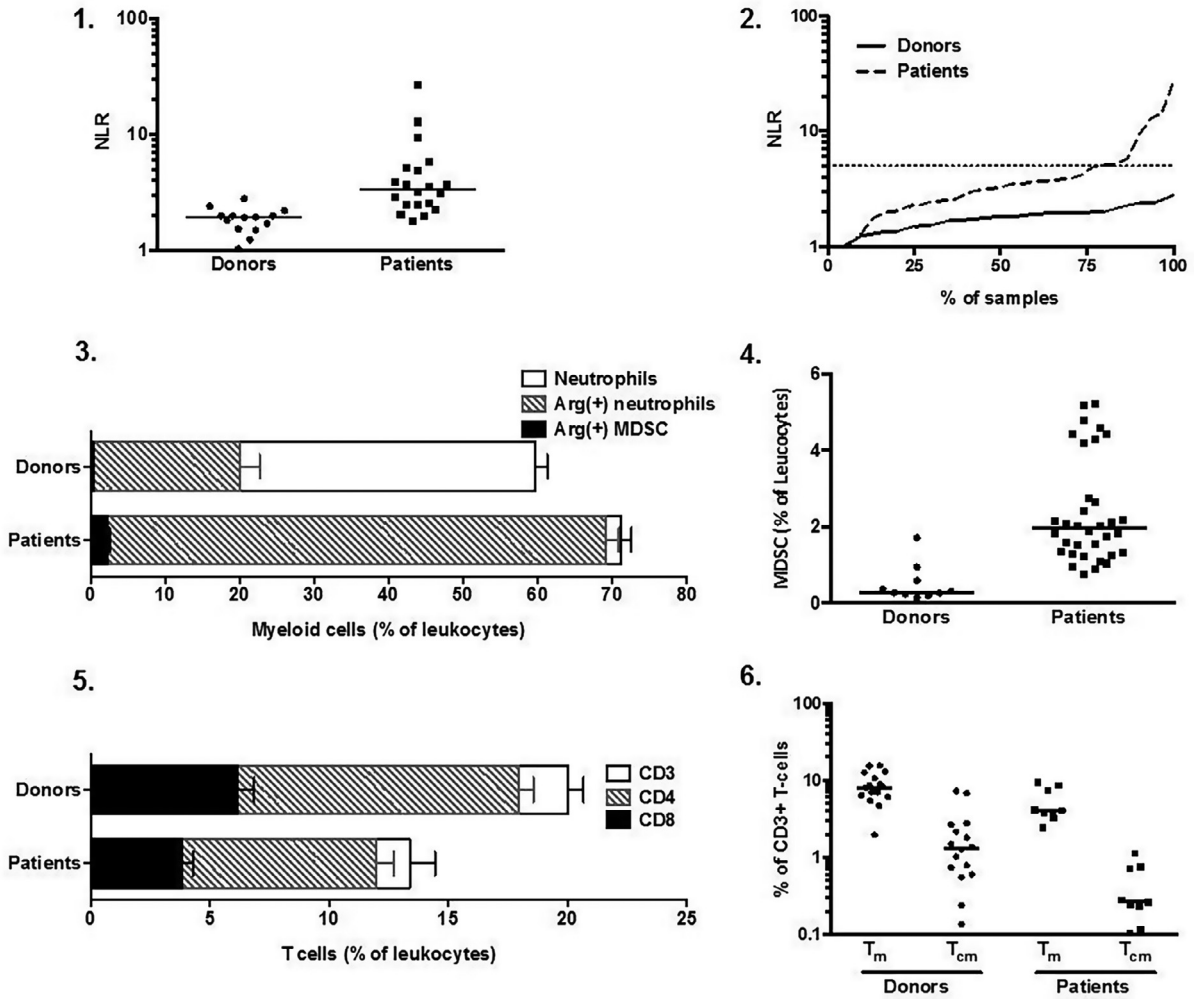

Fig. 1. Decoding NLR by flow cytometry in rectal cancer patients as compared with donors. NLR distribution is expressed respectively as dot plots (panel 1) or cumulative curves (panel 2); the composition of neutrophils (total, Arg^+ neutrophils and MDSC) is expressed as a percentage (panel 3), while distribution of Arg^+ MDSC is shown apart in panel 4; the composition of lymphocytes ($CD3^+$, $CD8^+$, and $CD4^+$ T cells) is expressed as a percentage (panel 5), while distribution of memory and central memory $CD8^+$ T cells, abbreviated as T_m and T_{cm} respectively, is shown apart in panel 6. MDSC, T_m and T_{cm} are phenotyped as $Arg^+Lin^-HLA\text{-}DR^{low}CD16^{low}CD33^+CD15^+$, $CD45RA^-CD27^+CD8^+$ and $CD45RA^-CD27^+CCR7^+$ $CD62L^+$ $CD8^+$ respectively. These data are a follow-up of our recently published observation [79].

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The Warburg effect has been historically described as the exacerbated glycolytic tumor metabolism that occurs even under well-oxygenated conditions, despite the fact that oxidative phosphorylation in mitochondria is a more efficient way to generate ATP [89]. Apparently, the serine-threonine kinase mTOR protein that senses the energy status of cells and more particularly the availability of nutrients, participates to the Warburg switch in tumor cells, a paradigm that may be expanded to T cells [90], as depicted in Fig. 2. The rapamycin-sensitive mTOR pathways operate mainly through the multi-protein complex 1 (mTORC1), which is conserved in a threefold sense. First, it is evolutionarily preserved from yeast all the way up to mammals. Second, its primary purpose is to guard cell survival in the event of energy deficit by inhibiting proliferation. Finally, the preserving function of mTOR is ensured by dominant constitutive negative regulators, like TSC1/2, AKT, AMPK and PRAS40. Upon activation with growth factors and/or cytokines, mTOR triggers glucose uptake and aerobic glycolysis – to produce the intermediate precursors essential for biomass growth, while blocking further pyruvate oxidation for the maximal ATP output within mitochondria [91]. Tumor cells frequently overexpress mTOR, thereby escaping from the growth arrest in any conditions including chronic hypoxia and nutrient starvation [92]. A similar escape likely holds true for T cells under chronic viral infections (EBV, CMV, HBV, HIV) and tumor-associated inflammation, which provide an array of growth-stimulating cytokines and provoke the overuse of CD45RO memory pools [80,93]. As a consequence, the age-related decay of pluripotent memory $CD8^+$ T cells that respond to CD28-mediated stimulation may be further aggravated despite that the circulating memory pool rises (an inflation effect) at the cost of naïve $CD28^+$ $CD57^-$ subsets [88,94]. This picture of a drained memory T-cell pool might be a possible explanation of the unsatisfied results of immunotherapy in CRC, given that CRC is commonly associated with chronic inflammation [95–97]. What are the possible mechanisms of mTOR-mediated T-cell proliferation/differentiation and what CD45RO-biased immunocorrective interventions will be available in the nearest future?

Of note, the metabolic check-points in T cells are similar to those in normal/cancer mammalian cells, and are reciprocally controlled by mTOR and AMPK - two opposed energy sensors/switches that put forward anabolism and catabolism respectively (Fig. 2) [24,98,99]. We talk here about an overall balance of anabolism versus catabolism rather than the switch-off/on, as both growing and quiescent cells require ATP supplied by catabolic reactions. In more detail, AMPK is activated by an increase in AMP/ATP ratio, which regulates oxidative phosphorylation and makes a transition toward the catabolic type of metabolism. In addition, AMPK inhibits mTORC1 thereby slowing down glycolysis and anabolic build-up of proteins, lipids and nucleotides. Alternatively, when ample amounts of energy and nutrients are available and both T-cell receptor and co-stimulatory signals are present, PI3 kinase is activated leading to the mTORC1-mediated induction of HIF-1α and Myc. Subsequently, metabolic reprogramming toward aerobic glycolysis is initiated while the transcriptional factors T-bet, BLIMP1, and STAT4 instruct $CD8^+$ T cells to differentiate into a $KLRG1^{hi}$ $IL\text{-}7R^{low}$ $CXCR3^{low}$ $CD62L^{low}$ phenotype, featured by an increased cytotoxicity against infection and tumor cells. Following the danger clearance, effector $CD8^+$ T cells reduce their dependence on glycolysis and gradually reset back to the catabolic state, a known marker of memory cells. Alongside, the T-cell phenotype changes toward a memory-type, characterized by down-regulation of KLRG1 and re-expression of CD62L/CCR7 and the IL-7 receptor. The transcriptional factors EOMES, BCL-6, and STAT3 further induce memory $CD8^+$ T cells to acquire a self-renewal capacity and longevity associated with the overexpression of anti-apoptotic proteins Bcl2 and Mcl-1. In this process, IL-7 is essential for the development and maintenance of memory T cells, whereas IL-15 primarily sustains their expansion [100]. Overall, the transition between the effector and memory functions in T cells is regu-

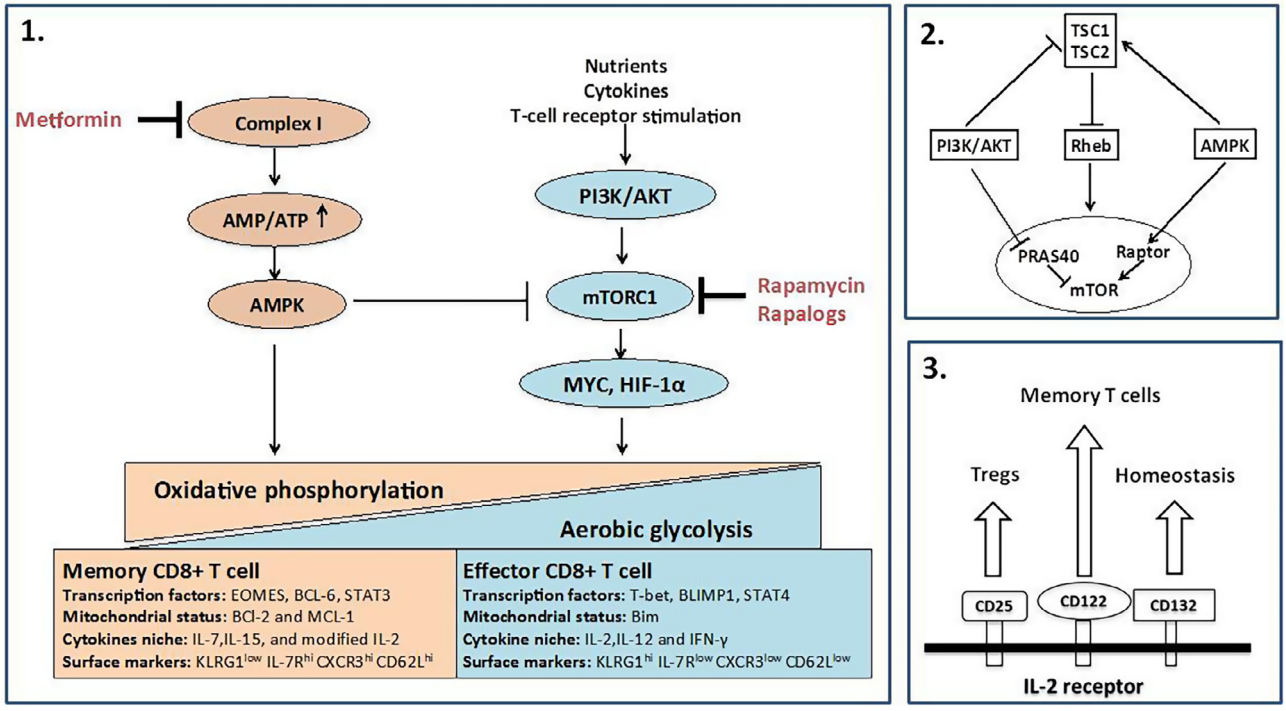

Fig. 2. mTOR and IL-2 pathways in T-cell differentiation. The cross talk of mTOR and AMPK pathways in T-cell differentiation and molecular targets for mTOR inhibitors (panel 1); the multi-protein complex mTOR in normal and cancer cells (panel 2); IL-2 receptor signaling to Tregs and memory T cells (panel 3). These simplified diagrams have been adapted after [24,28,98].

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lated at the coordinated levels of mTOR-driven glucose metabolism, transcription factors, mitochondrial status/apoptosis and cytokines [87].

While mTOR inhibition may favor the expansion of memory subsets at the cost of terminally differentiated effectors, a more specific cytokine signaling through IL-7, IL-15 or (modified) IL-2 is indispensable to shape the anti-tumor functionality of long-living central memory CD8+ cells. Thus CD45RO-biased immunotherapy could rely on two complementary types of intervention assigned to (a) a graded mTOR inhibition, either directly (rapamycin/rapalogs) or indirectly (metformin) and (b) an optimal cytokine niche that activates CD8+ T cells rather than CD4+ Tregs. In this regard, metformin and CD122-directed IL-2 complexes seem to be of special interest for future clinical trials in rectal cancer. Accumulating evidence suggests that the switch from glycolysis to fatty acid oxidation is a key process during the effector to memory cell transition, which involves the transition from a metabolic state governed by the mTOR signaling pathway to a metabolic state governed by the AMPK signaling pathway [24]. Metformin as an AMPK activator and the same time a mTOR inhibitor therefore stands a great potential to initiate the reprograming of effector T-cells to a memory phenotype [26]. In addition, after the transition, to efficiently replenish the memory T-cell pool, it is essential to boost the number with the help of cytokines that promote proliferation. In this context, CD122-directed IL-2 complexes are one of the best candidates due to the higher expression of CD122 on memory T-cell than on counterparts such as Tregs [101].

CD45-biased immunotherapy beyond immune checkpoints

Multifaceted mTOR inhibitors with immunocorrective properties

Several lines of evidence suggest that metformin, a drug of choice for the treatment of type II diabetes, offers great promise for cancer treatment and prevention, and may be repurposed for immunotherapeutic applications [23,102]. First, the recent meta-analysis of CRC incidence demonstrated a decreased risk ratio of 0.64 (0.54–0.76) for diabetes patients who did take metformin when compared with those not-taking this drug [103]. Second, three retrospective clinical studies revealed that CRC patients who use metformin as a part of their diabetic therapy have a significant survival advantage estimated by overall and cancer-specific mortality [104–106]. Specifically in rectal cancer, metformin users showed an improved pCR rate on univariate ($P = 0.05$) and multivariate ($P = 0.01$) analysis, leading to significantly increased disease-free survival ($P = 0.013$) when compared with other diabetic patients [106]. Third, about 10 on-going prospective phase II clinical trials are initiated since 2011 to explore whether metformin may improve therapy outcomes or lower CRC incidence in patients without diabetes. So far, the major focus on metformin in oncology is still directed to breast and prostate cancer [107–109], and only two phase II studies address neoadjuvant metformin in locally advanced rectal cancer with the primary endpoint being pCR (NCT02437656 and NCT03053544). Fourth, preclinical models suggest that the antitumor effect of metformin is most likely to be related to the inhibition of mTOR signaling pathways, which is triggered indirectly through targeting mitochondrial complex I and downstream AMPK activation [23]. This effect is similar to that of rapamycin, a direct powerful mTOR inhibitor, which is under investigation as an antitumor drug in clinical trials as well [110]. Currently, the second and third generation of rapalogs, e.g. ATP-competitive and bivalent mTOR inhibitors, are tested in clinical trials in a wide range of malignancies but the results are still awaited. Finally, a preclinical study illuminated how metformin can restore the functionality of lymphocytes in the tumor microenvironment through an effector-memory T-cell subset, which is responsible for tumor rejection [27]. We believe that metformin may be directly implemented into standard neoadjuvant chemoradiation in locally advanced rectal cancer, considering low if any toxicity of its chronic use. Despite that rapamycin shows a comparable restoration of memory T cells in mouse models [25,26], its clinical potential is less rationalized in the view of strong immunosuppressive effects exploited for organ transplantation [111]. On the other hand, metformin has been announced in the press as the first ever safe anti-aging drug to pursue life longevity, a remarkable medical event to be examined in coming 6-year clinical trials (NCT02432287). With these developments in mind, a phase II clinical trial is running (in our institution, EudraCT number: 2017-000814-50) for locally advanced cT3-4 rectal cancer, where metformin is combined with neoadjuvant chemoradiation to improve tumor radio/immunoresponse and patient outcome. Furthermore, the immunocorrecting properties of metformin in comparison with rapalogs are currently under preclinical investigation to support the next steps in CD45RO-biased immunotherapy.

IL-2 signaling in tumor surveillance versus escape

Among cytokines, IL-2, IL-7 and IL-15 are the most valuable candidates for tumor immunotherapy, and IL-2, the major T-cell growth factor, has been extensively studied in melanoma and renal cancer two decades ago. Unfortunately, severe side effects, including vascular leakage syndrome, hypotension and a preferential induction of Tregs, have been observed at high doses of IL-2 [29]. The breakthrough for this matter came from two sides, namely immunocomplexing and pegylation, which changed our understanding on the nature of IL-2/receptor interaction and signaling [28,112]. All three cytokines above share the γ-receptor chain (CD132) that in part explains their redundancy and the key role in lymphocyte homeostasis [113]. However, it are two other subunit chains – CD25 (IL-2Ra) and CD122 (IL-2Rb) – that create a variety of unique effects through the trimeric IL-2 receptor. Although CD25 binds IL-2 with low affinity (compared with di/trimers), its strong constitutive overexpression on Tregs enables these immunosuppressive cells to benefit from immunostimulation and eventually outperform T-cell cytotoxicity in favor of immunotolerance [29]. Therefore, the selective blocking of CD25-mediated signaling is critical in order to trigger memory T-cell expansion through CD122, by analogy to IL-15 that lacks CD25 signaling. On the experimental level, this effect can be achieved by the monoclonal antibody S4B6 that forms an immunocomplex with IL-2 and thereby stimulates memory $CD8^+$ T and NK cells without affecting Tregs [28]. Another elegant way for CD122-mediated immunostimulation is already one step forward in clinical trials, and based on the engineered IL-2 prodrug, NKTR-214, with 6 releasable polyethylene glycol (PEG) chains [112]. This modified IL-2 was well tolerated in mice and upon partial depegylation/activation induced durable antitumor immune responses linked to memory T-cell activation.

Conclusions

In summary, significant advancements in the management of locally advanced rectal cancer have occurred over the last decades, however, the risk of distant metastases remains an ongoing problem and the major obstacle to improve the survival rate. The cutting-edge blockade of immune checkpoints introduced a possibility of long-term survivors in immunogenic tumors, like melanoma, that may not be applicable to the majority of CRC due to low immunogenic mutation loading. In CRC, in situ memory T cells predict long-term oncological outcomes, mirroring the unique abil-

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ity of memory T cells to provide lifelong immune surveillance. With the increased understanding of the mechanisms that govern the formation of memory T cells, the generation of memory T cells becomes now one of the major focuses to treat chronic viral infections and cancer. In this context, metformin as a mTOR inhibitor is shown to reprogram the metabolism of T cells toward oxidative phosphorylation and thus aggravating the generation of memory T cells in preclinical settings, which is being validated in a running clinical trial in our institution. After the transition, memory T cells require a second trigger to maintain their expansion. The modified IL-2 (a CD122 receptor ligand) could be a good candidate due to its preferential capacity to bind to memory T cells. Their combinational effect in the frame of the treatment of rectal cancer requires further investigation; however there is a possibility that this approach might offer a new means to cope with unsatisfied distant control and survival. In addition, more efforts should be taken for a detailed immunoprofiling of rectal cancer to identify the high-risk subgroup of patients for immunotherapy, for example, the ratio of MDSC-to-memory T cells rather than basic NLR.

Acknowledgements

This work was supported by a grant from the Kom Op Tegen Kanker. The authors thank Prof. Dr. Guy Storme and Dr. Dirk Van den Berge for thoughtful and critical discussion.

Conflict of interest statement

The authors declare no potential conflict of interest.

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Please cite this article in press as: de Mey S et al. Potential of memory T cells in bridging preoperative chemoradiation and immunotherapy in rectal cancer. Radiother Oncol (2018), https://doi.org/10.1016/j.radonc.2018.04.003