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MARKERS SELECTIVELY DEREGULATED IN TUMOR-INFILTRATING REGULATORY T CELLS

Abstract

The present invention discloses a number of markers selectively deregulated in tumor-infiltrating regulatory T cells. The invention relates to molecules able to modulate the expression and/or function of at least one such marker for use in the prevention and/or treatment of the tumor. Preferably the molecule specifically binds to the marker and induces antibody-dependent cell-mediated cytotoxicity (ADCC). The invention further relates to a molecule able to modulate the expression and/or function of at least one such marker for use in a method for in vivo depleting tumor-infiltrating regulatory T cell in a subject, or for use in a method to enhance tumor immunity in a subject. Corresponding pharmaceutical compositions are also contemplated.

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Background/Summary

RELATED APPLICATIONS [0001] This application is a continuation of U.S. application Ser. No. 18/295,965, filed Apr. 5, 2023, which is a continuation of U.S. application Ser. No. 16/301,805, filed Nov. 15, 2018, which is a national stage filing under 35 U.S.C. § 371 of International Application Serial No. PCT/EP2017/061642, filed May 15, 2017, the contents of each of which is incorporated herein by reference in their entirety.

REFERENCE TO AN ELECTRONIC SEQUENCE LISTING
[0002] The contents of the electronic sequence listing (C158570000US02-SEQ-JRV.xml; Size: 953,347 bytes; and Date of Creation: Jan. 24, 2025) is herein incorporated by reference in its entirety.

FIELD OF THE INVENTION
[0003] The present invention relates to a molecule able to modulate the expression and/or function of at least one marker that is selectively deregulated in tumor-infiltrating regulatory T cell or to a molecule capable of specifically binding to at least one marker that is selectively deregulated in tumor-infiltrating regulatory T cell and inducing antibody-dependent cell-mediated cytotoxicity (ADCC) for use in the prevention and/or treatment of cancer or for use in a method for in vivo depleting tumor-infiltrating regulatory T cell in a subject or for use in a method to enhance tumor immunity in a subject and relative pharmaceutical composition.

BACKGROUND OF THE INVENTION
[0004] The combination of genetic mutations and epigenetic modifications that are peculiar to all tumors generate antigens that T and B lymphocytes can use to specifically recognize tumor cells (Jamal-Hanjani et al., 2013). It is increasingly clear that T lymphocytes recognizing tumor derived peptides presented by major histocompatibility complex (MHC) molecules play a central role in immunotherapy and in conventional chemo-radiotherapy of cancer (Galluzzi et al., 2015). In fact, anti-tumor T cell responses arise in cancer patients but are disabled upon tumor progression by suppressive mechanisms triggered by the interplay between malignant cells and the tumor microenvironment (Munn and Bronte, 2015). The tumor-dependent immunosuppressive mechanisms depend on the integrated action of infiltrating leukocytes and lymphocytes that upregulate a range of modulatory molecules, collectively called immune checkpoints, whose function is only partially characterized (Pardoll, 2012). Therefore, the search for agonists of co-

stimulatory complexes or antagonists of inhibitory molecules to potentiate antigen-specific T cell responses is a primary goal of current anti-tumor research (Sharma and Allison, 2015; Zitvogel et al., 2013). Indeed, clinical trials have unequivocally shown that the blockade of immune checkpoints unleashes the spontaneous anti-tumor immune responses in such a powerful way that it has created a paradigm shift in cancer therapy (Sledzinska et al., 2015; Topalian et al., 2015).
[0005] Amongst the immune checkpoints targeted by blocking strategies, CTLA-4 has been one of the first to be translated into therapeutic applications.
[0006] Anti-CTLA-4 monoclonal antibodies (mAb) showed remarkable success in metastatic melanoma, and more recently in non-small-cell lung cancer, prostate cancer, renal cell carcinoma, urothelial carcinoma and ovarian cancer (Carthon et al., 2010; Hodi et al., 2010; van den Eertwegh et al., 2012; Yang et al., 2007). However, the fraction of patients that do not respond remains high, prompting a deeper investigation of the mechanisms underpinning the modulation of immune responses by tumors. Recent experimental evidence showed that anti-CTLA-4 mAb efficacy depends on FcγR mediated depletion of CD4.sup.+ regulatory T cells (Treg cells) within the tumor microenvironment (Peggs et al., 2009; Selby et al., 2013; Simpson et al., 2013; Twyman-Saint Victor et al., 2015). Treg cells, which are physiologically engaged in the maintenance of immunological self-tolerance and immune homeostasis (Josefowicz et al., 2012; Sakaguchi et al., 2008), are potent suppressors of effector cells and are found at high frequencies in various types of cancers (Fridman et al., 2012; Nishikawa and Sakaguchi, 2010). Interestingly, Treg cells adapt their transcriptional program to the various cytokines to which they are exposed in the inflammatory milieu (Campbell and Koch, 2011). This versatility is controlled by transcription factors generally associated with the differentiation of other effector CD4+ T cell subsets, resulting in various Treg cell populations with unique features and immunomodulatory functions (Duhen et al., 2012; Geginat et al., 2014). Moreover, Treg cells infiltrating non-lymphoid tissues are reported to exhibit unique phenotypes and transcriptional signatures, as they can display functions beyond their well-established suppressive roles, such as metabolic modulation in adipose tissue (Cipolletta et al., 2012) or regulation of tissue repair in skeletal muscle (Burzyn et al., 2013) and in lung tissue (Arpaia et al., 2015).
[0007] Treg cells depletion has been reported to increase anti-tumor specific immune responses and to reduce tumor burden (Marabelle et al., 2013; Teng et al., 2010; Walter et al., 2012).
[0008] Although promising clinical results have been achieved with Treg cell depleting strategies, some relevant issues are to be addressed, for a safer, more effective and wider clinical application of these therapies. First, severe autoimmunity can occur following systemic Treg cells depletion (Nishikawa and Sakaguchi, 2010), which could be avoided if selective depletion of tumor infiltrating Treg cells were feasible. A second issue concerns the specificity of targeting, indeed Treg cells share with effector lymphocytes most of the molecules targeted for therapy, which can possibly deplete also the tumor-specific effector cells. Therefore, the molecular characterization of Treg cells at different tumor sites should help to better define therapeutic targets through a better description of their signature molecules and of the network that regulates Treg cell functions in the tumor microenvironment.
[0009] Non-small-cell lung cancer (NSCLC) and colorectal cancer (CRC) are the two most frequent cancers in both genders (Torre et al., 2015). NSCLC has the worst prognosis due to its high mortality rate even in early stages. Although CRC survival rate is highly dependent on the tumor stage at diagnosis, about 50% of patients will progress to metastatic cancer (Gonzalez-Pons and Cruz-Correa, 2015). Both tumors have been targeted with therapies based on monoclonal antibodies to checkpoint inhibitors, but the outcomes were different. While remarkable clinical success has been obtained in NSCLC, evidence of durable response in CRC is scarce with the exception of mismatch repair-deficient CRC lesions (Jacobs et al., 2015; Kroemer et al., 2015; Le et al., 2015).
[0010] Then there is still need for agents that target tumor infiltrating Treg cells for the treatment and/or prevention of cancer.

SUMMARY OF THE INVENTION

[0011] Tumor-infiltrating regulatory T lymphocytes (Treg) can suppress effector T cells specific for tumor antigens. Since new anti-cancer immunotherapies aim at unleashing effector T cells by targeting immune-checkpoints, deeper molecular definitions of tumor-infiltrating-lymphocytes could offer new therapeutic opportunities. Transcriptomes of T helper 1(Th1), Th17 and Treg cells infiltrating colorectal or non-small-cell lung cancers were compared to transcriptomes of the same subsets from normal tissues, and validated at the single cell level. The inventors found tumor-infiltrating Treg cells are highly suppressive, upregulate several immune-checkpoints, and express on the cell surface specific signature molecules such as interleukin-1 receptor 2 (IL1R2), programmed death (PD)-1 Ligand1, PD-1 Ligand2, and CCR8 chemokine which were not previously described on Treg cells. Remarkably, high expression in whole tumor samples of Treg signature genes, such as LAYN, MAGEH1 or CCR8, correlated with poor prognosis. The invention provides new insights into the molecular identity and functions of human tumor-infiltrating Treg cells, and define new potential targets for tumor immunotherapy.

[0012] In the present invention, the inventors provide a comprehensive transcriptome analysis of human CD4.sup.+ Treg cells and effector cells (Th1 and Th17) infiltrating NSCLC or CRC and their matched normal tissues.

[0013] Inventors defined molecular signatures of tumor-infiltrating Treg cells in these two cancer types and confirmed the relevance of these signatures by single-cell analyses. These data could help a better understanding of Treg functional role at tumor sites and pave the way to the identification of therapeutic targets for more specific and safer modulation of Treg cells in cancer therapy.

[0014] The inventors' findings provide new insights on the inhibitory mechanisms of Treg cells and offer precise targets for cancer immunotherapy.

[0015] Then the present invention provides a molecule able to modulate the expression and/or function of at least one marker that is selectively deregulated in tumor-infiltrating regulatory T cells for use in the prevention and/or treatment of said tumor.

[0016] Preferably, the molecule according to the invention is capable of specifically binding to said at least one marker and inducing antibody-dependent cell-mediated cytotoxicity (ADCC).

[0017] Said molecule is preferably able to selectively deplete tumor-infiltrating regulatory T cells.

[0018] Said molecule is preferably selected from the group consisting of: [0019] a) an antibody or a fragment thereof; [0020] b) a polypeptide; [0021] c) a small molecule; [0022] d) a polynucleotide coding for said antibody or polypeptide or a functional derivative thereof; [0023] e) a polynucleotide, such as antisense construct, antisense oligonucleotide, RNA interference construct or siRNA, [0024] e) a vector comprising or expressing the polynucleotide as defined in d) or e); [0025] f) a host cell genetically engineered expressing said polypeptide or antibody or comprising the polynucleotide as defined in d) or e).

[0026] Preferably, the marker is selected from the group consisting of at least one marker disclosed in the following Table VIII.

TABLE-US-00001 TABLE VIII MARKER ENTREZ_ID NAME ENSEMBL_release87 release108 FUCA2 ENSG00000001036 2519 ICA1 ENSG00000003147 3382 TTC22 ENSG00000006555 55001 COX10 ENSG00000006695 1352 IL32 ENSG00000008517 9235 ETV7 ENSG00000010030 51513 ATP2C1 ENSG00000017260 27032 FAS ENSG00000026103 355 ARNTL2 ENSG00000029153 56938 IKZF2 ENSG00000030419 22807 PEX3 ENSG00000034693 8504 MAT2B ENSG00000038274 27430 TSPAN17 ENSG00000048140 26262 COL9A2 ENSG00000049089 1298 TNFRSF9 ENSG00000049249 3604 FOXP3 ENSG00000049768 50943 NFE2L3 ENSG00000050344 9603 LIMA1 ENSG00000050405 51474 TNIP3 ENSG00000050730 79931 LY75 ENSG00000054219 4065 ZNF280C ENSG00000056277 55609 YIPF1 ENSG00000058799 54432 NFYC ENSG00000066136 4802 ISOC1 ENSG00000066583 51015 PHKA1 ENSG00000067177 5255 ACSL4 ENSG00000068366 2182 MAST4 ENSG00000069020 375449 LMCD1 ENSG00000071282 29995 TFRC ENSG00000072274 7037 PANX2

ENSG00000073150 56666 FNDC3B ENSG00000075420 64778 REXO2 ENSG00000076043 25996 TP73 ENSG00000078900 7161 LXN ENSG00000079257 56925 CEACAM1 ENSG00000079385 634 IL12RB2 ENSG00000081985 3595 GSK3B ENSG00000082701 2932 TDRD3 ENSG00000083544 81550 RRAGB ENSG00000083750 10325 STARD7 ENSG00000084090 56910 SSH1 ENSG00000084112 54434 NCOA1 ENSG00000084676 8648 MGST2 ENSG00000085871 4258 ACOX3 ENSG00000087008 8310 AURKA ENSG00000087586 6790 TPX2 ENSG00000088325 22974 ANKRD10 ENSG00000088448 55608 FKBP1A ENSG00000088832 2280 SIRPG ENSG00000089012 55423 BIRC5 ENSG00000089685 332 RGS1 ENSG00000090104 5996 DPYSL2 ENSG00000092964 1808 WHRN ENSG00000095397 25861 CENPM ENSG00000100162 79019 SEPT3 ENSG00000100167 55964 NCF4 ENSG00000100365 4689 CSF2RB ENSG00000100368 1439 IL2RB ENSG00000100385 3560 CNIH1 ENSG00000100528 10175 ZMYND8 ENSG00000101040 23613 MAP1LC3A ENSG00000101460 84557 PIGU ENSG00000101464 128869 NXT2 ENSG00000101888 55916 SMS ENSG00000102172 6611 NDFIP2 ENSG00000102471 54602 ACP5 ENSG00000102575 54 NFAT5 ENSG00000102908 10725 CYB5B ENSG00000103018 80777 IL21R ENSG00000103522 50615 LAPTM4B ENSG00000104341 55353 IL7 ENSG00000104432 3574 NCALD ENSG00000104490 83988 ERI1 ENSG00000104626 90459 EBI3 ENSG00000105246 10148 PLA2G4C ENSG00000105499 8605 CDK6 ENSG00000105810 1021 HOXA1 ENSG00000105991 3198 GLCCI1 ENSG00000106415 113263 MINPP1 ENSG00000107789 9562 ACTA2 ENSG00000107796 59 WSB1 ENSG00000109046 26118 CLNK ENSG00000109684 116449 HTATIP2 ENSG00000109854 10553 CTSC ENSG00000109861 1075 VWA5A ENSG00000110002 4013 DCPS ENSG00000110063 28960 SLC35F2 ENSG00000110660 54733 FOXM1 ENSG00000111206 2305 RAD51AP1 ENSG00000111247 10635 RASAL1 ENSG00000111344 8437 VDR ENSG00000111424 7421 FAM184A ENSG00000111879 79632 DNPH1 ENSG00000112667 10591 KIF20A ENSG00000112984 10112 SEC24A ENSG00000113615 10802 KAT2B ENSG00000114166 8850 PPM1G ENSG00000115241 5496 IL1R2 ENSG00000115590 7850 IL1R1 ENSG00000115594 3554 IL1RL2 ENSG00000115598 8808 IL1RL1 ENSG00000115602 9173 UXS1 ENSG00000115652 80146 SLC25A12 ENSG00000115840 8604 THADA ENSG00000115970 63892 PARK7 ENSG00000116288 11315 LEPR ENSG00000116678 3953 GADD45A ENSG00000116717 1647 KIF14 ENSG00000118193 9928 MREG ENSG00000118242 55686 HSDL2 ENSG00000119471 84263 FLVCR2 ENSG00000119686 55640 CD274 ENSG00000120217 29126 SOCS2 ENSG00000120833 8835 TNFRSF8 ENSG00000120949 943 RDH10 ENSG00000121039 157506 LAX1 ENSG00000122188 54900 TWIST1 ENSG00000122691 7291 ZWINT ENSG00000122952 11130 CIT ENSG00000122966 11113 ACOT9 ENSG00000123130 23597 IKZF4 ENSG00000123411 64375 HJURP ENSG00000123485 55355 METTL8 ENSG00000123600 79828 TOX2 ENSG00000124191 84969 GTSF1L ENSG00000124196 149699 SOX4 ENSG00000124766 6659 TM9SF2 ENSG00000125304 9375 HS3ST3B1 ENSG00000125430 9953 EML2 ENSG00000125746 24139 MGME1 ENSG00000125871 92667 IGFLR1 ENSG00000126246 79713 DLGAP5 ENSG00000126787 9787 HIVEP3 ENSG00000127124 59269 LRRC61 ENSG00000127399 65999 TST ENSG00000128311 7263 STRIP2 ENSG00000128578 57464 MYO5C ENSG00000128833 55930 FOXA1 ENSG00000129514 3169 ITFG1 ENSG00000129636 81533 KLHDC7B ENSG00000130487 113730 TRAF3 ENSG00000131323 7187 MCCC2 ENSG00000131844 64087 GRSF1 ENSG00000132463 2926 SYT11 ENSG00000132718 23208 SLC41A1 ENSG00000133065 254428 ATP13A3 ENSG00000133657 79572 MICAL2 ENSG00000133816 9645 IL2RA ENSG00000134460 3559 CABLES1 ENSG00000134508 91768 RFK ENSG00000135002 55312 HAVCR2 ENSG00000135077 84868 CGA ENSG00000135346 1081 FAIM2 ENSG00000135472 23017 EGLN1 ENSG00000135766 54583 ARHGEF4 ENSG00000136002 50649 SLC41A2 ENSG00000136052 84102 FLNB ENSG00000136068 2317 RCBTB1 ENSG00000136144 55213 TMOD1 ENSG00000136842 7111 TPMT

ENSG00000137364 7172 CASP1 ENSG00000137752 834 NUSAP1 ENSG00000137804 51203 ADAM10 ENSG00000137845 102 ZNF280D ENSG00000137871 54816 HADHB ENSG00000138029 3032 CEP55 ENSG00000138180 55165 ENTPD1 ENSG00000138185 953 NAB1 ENSG00000138386 4664 HECW2 ENSG00000138411 57520 CD27 ENSG00000139193 939 CDH24 ENSG00000139880 64403 RAB15 ENSG00000139998 376267 ETFA ENSG00000140374 2108 KSR1 ENSG00000141068 8844 PCTP ENSG00000141179 58488 SECTM1 ENSG00000141574 6398 EVA1B ENSG00000142694 55194 WDTC1 ENSG00000142784 23038 CTTNBP2NL ENSG00000143079 55917 CASQ1 ENSG00000143318 844 SNAP47 ENSG00000143740 116841 STAC ENSG00000144681 6769 ARL6IP5 ENSG00000144746 10550 ADPRH ENSG00000144843 141 PAM ENSG00000145730 5066 RNF145 ENSG00000145860 153830 TTBK1 ENSG00000146216 84630 TMEM140 ENSG00000146859 55281 CHST7 ENSG00000147119 56548 CHRNA6 ENSG00000147434 8973 MKI67 ENSG00000148773 4288 PTPRJ ENSG00000149177 5795 ZC3H12C ENSG00000149289 85463 NCAM1 ENSG00000149294 4684 INPP1 ENSG00000151689 3628 JAKMIP1 ENSG00000152969 152789 GTF3C6 ENSG00000155115 112495 RHOC ENSG00000155366 389 SLC16A1 ENSG00000155380 6566 BATF ENSG00000156127 10538 CXCL13 ENSG00000156234 10563 SH3RF2 ENSG00000156463 153769 NPTN ENSG00000156642 27020 CCNB2 ENSG00000157456 9133 RNF207 ENSG00000158286 388591 AHCYL2 ENSG00000158467 23382 PTGIR ENSG00000160013 5739 CALM3 ENSG00000160014 808 TMPRSS3 ENSG00000160183 64699 FCRL3 ENSG00000160856 115352 PAQR4 ENSG00000162073 124222 ZG16B ENSG00000162078 124220 JAK1 ENSG00000162434 3716 DIRAS3 ENSG00000162595 9077 ACTG2 ENSG00000163017 72 SGPP2 ENSG00000163082 130367 NEURL3 ENSG00000163121 93082 CTLA4 ENSG00000163599 1493 ICOS ENSG00000163600 29851 RYBP ENSG00000163602 23429 KIF15 ENSG00000163808 56992 TMEM184C ENSG00000164168 55751 C5orf63 ENSG00000164241 401207 PTTG1 ENSG00000164611 9232 MELK ENSG00000165304 9833 FAAH2 ENSG00000165591 158584 PRDX3 ENSG00000165672 10935 HPRT1 ENSG00000165704 3251 CACNB2 ENSG00000165995 783 TPP1 ENSG00000166340 1200 AKIP1 ENSG00000166452 56672 ACAA2 ENSG00000167315 10449 GNG8 ENSG00000167414 94235 GNG4 ENSG00000168243 2786 CX3CR1 ENSG00000168329 1524 AHCYL1 ENSG00000168710 10768 TSPAN5 ENSG00000168785 10098 PGM2 ENSG00000169299 55276 CRADD ENSG00000169372 8738 UGP2 ENSG00000169764 7360 ZNF282 ENSG00000170265 8427 GLB1 ENSG00000170266 2720 SMAD1 ENSG00000170365 4086 SPATA24 ENSG00000170469 202051 PRKCDBP ENSG00000170955 112464 TADA3 ENSG00000171148 10474 RBKS ENSG00000171174 64080 NETO2 ENSG00000171208 81831 LRG1 ENSG00000171236 116844 FAM98B ENSG00000171262 283742 CHST11 ENSG00000171310 50515 ECEL1 ENSG00000171551 9427 BCL2L1 ENSG00000171552 598 MALT1 ENSG00000172175 10892 ZMAT3 ENSG00000172667 64393 CORO1B ENSG00000172725 57175 CYP7B1 ENSG00000172817 9420 HPSE ENSG00000173083 10855 VANGL1 ENSG00000173218 81839 GLRX ENSG00000173221 2745 TRIB1 ENSG00000173334 10221 CD7 ENSG00000173762 924 HAP1 ENSG00000173805 9001 FBXO45 ENSG00000174013 200933 CHST2 ENSG00000175040 9435 RMI2 ENSG00000175643 116028 SLC35E3 ENSG00000175782 55508 ZBTB38 ENSG00000177311 253461 ZBED2 ENSG00000177494 79413 PARD6G ENSG00000178184 84552 GLDC ENSG00000178445 2731 AKAP5 ENSG00000179841 9495 CCR8 ENSG00000179934 1237 PAK2 ENSG00000180370 5062 YIPF6 ENSG00000181704 286451 TIGIT ENSG00000181847 201633 CREB3L2 ENSG00000182158 64764 XKRX ENSG00000182489 402415 CADM1 ENSG00000182985 23705 LHFP ENSG00000183722 10186 CSF1 ENSG00000184371 1435 PTP4A3 ENSG00000184489 11156 CDCA2 ENSG00000184661 157313 OSBP2 ENSG00000184792 23762 METTL7A ENSG00000185432 25840 SPATC1 ENSG00000186583 375686 TNFRSF4 ENSG00000186827 7293 TNFRSF18 ENSG00000186891 8784 TMPRSS6

ENSG00000187045 164656 GCNT1 ENSG00000187210 2650 MAGEH1 ENSG00000187601 28986 NHS ENSG00000188158 4810 IL17REL ENSG00000188263 400935 ADAT2 ENSG00000189007 134637 NEMP2 ENSG00000189362 100131211 SPATS2L ENSG00000196141 26010 NTNG2 ENSG00000196358 84628 MYL6B ENSG00000196465 140465 ARHGEF12 ENSG00000196914 23365 MAP3K5 ENSG00000197442 4217 PDGFA ENSG00000197461 5154 PDCD1LG2 ENSG00000197646 80380 TOR4A ENSG00000198113 54863 HIBCH ENSG00000198130 26275 ZNF334 ENSG00000198185 55713 NTRK1 ENSG00000198400 4914 TMA16 ENSG00000198498 55319 WDHD1 ENSG00000198554 11169 FAM19A2 ENSG00000198673 338811 F5 ENSG00000198734 2153 GK ENSG00000198814 2710 INPP5F ENSG00000198825 22876 LAYN ENSG00000204381 143903 CARD16 ENSG00000204397 114769 TBC1D8 ENSG00000204634 11138 CD177 ENSG00000204936 57126 LEPROT ENSG00000213625 54741 SEC14L6 ENSG00000214491 730005 TRIM16 ENSG00000221926 10626 LTA ENSG00000226979 4049 PROB1 ENSG00000228672 389333 AF165138.7 ENSG00000243440 NA USP51 ENSG00000247746 158880 CARD17 ENSG00000255221 440068 DOC2B ENSG00000272636 8447 C17orf96 ENSG00000273604 100170841 SSTR3 ENSG00000278195 6753 AC019206.1 ENSG00000279229 NA

wherein each of said marker name is characterized by “Ensembl gene id” and includes all of therein disclosed isoform protein sequences.

[0027] Each gene of table VIII is characterized by its Ensembl Gene accession number (ENSG), retrievable in the public database EnsEMBL (http://www.ensembi.orcl) and by its Entrez Gene ID, retrievable in the public database NCBI (https://www.ncbi.nim.nih.gov/), if present.

[0028] Preferably the marker is selected from the group consisting of: a transmembrane protein, a cytokine, an epigenetic factor, a kinase phosphatase or a transcription factor.

[0029] More preferably, the marker is a transmembrane protein selected from the group of SEQ ID NO:1-661, even more preferably, the marker is selected from the group consisting of: LAYN (SEQ ID NOs:1-9), CCR8 (SEQ ID Nos:10-11), IL21R (SEQ ID Nos: 12-14), IL1 R2 (SEQ ID Nos:206-209), LY75 (SEQ ID NO: 78), SIRPG (SEQ ID Nos:122-126), CD177 (SEQ ID Nos:651-653), CD7 (SEQ ID Nos:549-554), FCRL3 (SEQ ID Nos:452-457), CADM1 (SEQ ID Nos: 570-583), NTNG2 (SEQ ID Nos:621-622), CSF2RB (SEQ ID Nos:134-137), SECTMI (SEQ ID Nos: 349-356), TSPAN5 (SEQ ID Nos:497-503), TMPRSS3 (SEQ ID Nos:448-451), TMPRSS6 (SEQ ID Nos:605-611), METTL7A (SEQ ID Nos:600-604), THADA (SEQ ID Nos: 237), NDFIP2 (SEQ ID Nos:148-151), CHRNA6 (SEQ ID Nos:392-394), or from the group consisting of:

TABLE-US-00002 LAYN (SEQ ID NOS: 1-9

[>ENSG00000204381_ENST00000375614_ENSP00000364764_LAYN

MRPGTALQAVLLAVLLVGLRAATGRLLSGQPVCRGGTQRPCYKVIYFHDTSRRLNFEEAKEACR
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WYVDEPSCGSEVCVVMYHQPSAPAGIGGPYMFQWNDDRCNMKNNFICKYSDEKPAVPSREAE
GEETELTTPVLPEETQEEDAKKTFKESREAALNLAYILIPSIPLLLLLVVTTVVCWVWICRKRKRE
QPDPSTKKQHTIWPSPHQGNSPDLEVYNVIRKQSEADLAETRPDLKNISFRVCSGEATPDDMSCD
YDNMAVNPSESGFVTLVSVESGFVTNDIYEFSPDQMGRSKESGWVENEIYGY* (SEQ
ID NO: 1) >ENSG00000204381_ENST00000375615_ENSP00000364765_LAYN
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EEAKEACRRDGGQLVSIESEDEQKLIEKFIENLLPSDGDFWIGLRRREEKQSNSTACQDLYAWTD
GSISQFRNWYVDEPSCGSEVCVVMYHQPSAPAGIGGPYMFQWNDDRCNMKNNFICKYSDEKPA
VPSREAEGEETELTTPVLPEETQEEDAKKTFKESREAALNLAYILIPSIPLLLLLVVTTVVCWVWIC
RKRKREQPDPSTKKQHTIWPSPHQGNSPDLEVYNVIRKQSEADLAETRPDLKNISFRVCSGEATP
DDMSCDYDNMAVNPSESGFVTLVSVESGFVTNDIYEFSPDQMGRSKESGWVENEIYGY*
(SEQ ID NO: 2) >ENSG00000204381_ENST00000436913_ENSP00000392942_LAYN
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DAKKTFKESREAALNLAYILIPSIPLLLLLVVTTVVCWVWICRKRKREQPDPSTKKQHTIWPSPHQ

GNSPDLEVYNVIRKQSEADLAETRPDLKNISFRVCSGEATPDDMSCDYDNMAVNPSESGFVTLV
SVESGFVTNDIYEFSPDQMGRSKESGWVENEIYGY* (SEQ ID NO: 3)
>ENSG00000204381_ENST00000525126_ENSP00000434328_LAYN
MRPGTALQAVLLAVLLVGLRAATGRLLSASDLDLRGGQPVCRGGTQRPCYKVIYFHDTSRRLNF
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GSISQFRNWYVDEPSCGSEVCVVMYHQPSAPAGIGGPYMFQWNDDRCNMKNNFICKYSDEKPA
VPSREAEGEETELTTPVLPEETQEEDAKKTFKESREAALNLAYILIPSIPLLLLLVVTTVVCWVWIC
RKRQKTGAARP* (SEQ ID NO: 4)
>ENSG00000204381_ENST00000525866_ENSP00000434300_LAYN
MRPGTALQAVLLAVLLVGLRAATGRLLSGQPVCRGGTQRPCYKVIYFHDTSRRLNFEEAKEACR
RDGGQLVSIESEDEQKLIEKFIENLLPSDGDFWIGLRRREEKQSNSTACQDLYAWTDGSISQFRET
SSSF* (SEQ ID NO: 5)
>ENSG00000204381_ENST00000528924_ENSP00000486561_LAYN
MVTSGLGSGGVRRNKAIAQPARTFMLGLMAAYHNLEKPAVPSREAEGEETELTTPVLPEETQEE
DAKKTFKESREAALNLAYILIPSIPLLLLLVVTTVVCWVWICRK (SEQ ID NO: 6)
>ENSG00000204381_ENST00000530962_ENSP00000431627_LAYN
MYHQPSAPAGIGGPYMFQWNDDRCNMKNNFICKYSDEKPAVPSREAEGEETELTTPVLPEETQE
EDAKKTFKESREAALNLAYILIPSIPLLLLLVVTTVVCWVWICRK (SEQ ID NO: 7)
>ENSG00000204381_ENST00000533265_ENSP00000434972_LAYN
MRPGTALQAVLLAVLLVGLRAATGRLLSGQPVCRGGTQRPCYKVIYFHDTSRRLNFEEAKEACR
RDGGQLVSIESEDEQKLIEKFIENLLPSDGDFWIGLRRREEKQSNSTACQDLYAWTDGSISQFRN
WYVDEPSCGSEVCVVMYHQPSAPAGIGGPYMFQWNDDRCNMKNNFICKYSDEKPAVPSREAE
GEETELTTPVLPEETQEEDAKKTFKESREAALNLAYILIPSIPLLLLLVVTTVVCWVWICRKRQKT
GAARP* (SEQ ID NO: 8)
>ENSG00000204381_ENST00000533999_ENSP00000432434_LAYN
MYHQPSAPAGIGGPYMFQWNDDRCNMKNNFICKYSDEKPAVPSREAEGE (SEQ ID
NO: 9)]), CCR8 (SEQ ID Nos:10-11
[>ENSG00000179934_ENST00000326306_ENSP00000326432_CCR8
MDYTLDLSVTTVTDYYYPDIFSSPCDAELIQTNGKLLLAVFYCLLFVFSLLGNSLVILVLVVCKKL
RSITDVYLLNLALSDLLFVFSFPFQTYYLLDQWVFGTVMCKVVSGFYYIGFYSSMFFITLMSVDR
YLAVVHAVYALKVRTIRMGTTLCLAVWLTAIMATIPLLVFYQVASEDGVLQCYSFYNQQTLKW
KIFTNFKMNILGLLIPFTIFMFCYIKILHQLKRCQNHNKTKAIRLVLIVVIASLLFWVPFNVVLFLTS
LHSMHILDGCSISQQLTYATHVTEIISFTHCCVNPVIYAFVGEKFKKHLSEIFQKSCSQIFNYLGRQ
MPRESCEKSSSCQQHSSRSSSVDYIL* (SEQ ID NO: 10)
>ENSG00000179934_ENST00000414803_ENSP00000390104_CCR8
MDYTLDLSVTTVTDYYYPDIFSSPCDAELIQTNDLLSAGPVGVWDCNVQSGVWLLLHWLLQQH
VFHHPHECGQVPGCCPCRVCPKGEDDQDGHNAVPGSMANRHYGYHPIASVLPSGL*
(SEQ ID NO: 11)]), IL21R (SEQ ID Nos: 12-14
[>ENSG00000103522_ENST00000337929_ENSP00000338010_IL21R
MPRGWAAPLLLLLLQGGWGCPDLVCYTDYLQTVICILEMWNLHPSTLTLTWQDQYEELKDEAT
SCSLHRSAHNATHATYTCHMDVFHFMADDIFSVNITDQSGNYSQECGSFLLAESIKPAPPFNVTV
TFSGQYNISWRSDYEDPAFYMLKGKLQYELQYRNRGDPWAVSPRRKLISVDSRSVSLLPLEFRK
DSSYELQVRAGPMPGSSYQGTWSEWSDPVIFQTQSEELKEGWNPHLLLLLLLVIVFIPAFWSLKT
HPLWRLWKKIWAVPSPERFFMPLYKGCSGDFKKWVGAPFTGSSLELGPWSPEVPSTLEVYSCHP
PRSPAKRLQLTELQEPAELVESDGVPKPSFWPTAQNSGGSAYSEERDRPYGLVSIDTVTVLDAEG
PCTWPCSCEDDGYPALDLDAGLEPSPGLEDPLLDAGTTVLSCGCVSAGSPGLGGPLGSLLDRLKP
PLADGEDWAGGLPWGGRSPGGVSESEAGSPLAGLDMDTFDSGFVGSDCSSPVECDFTSPGDEGP
PRSYLRQWVVIPPPLSSPGPQAS* (SEQ ID NO: 12)
>ENSG00000103522_ENST00000395754_ENSP00000379103_IL21R
MPRGWAAPLLLLLLQGGWGCPDLVCYTDYLQTVICILEMWNLHPSTLTLTWQDQYEELKDEAT

SCSLHRSAHNATHATYTCHMDVFHFMADDIFSVNITDQSGNYSQECGSFLLAESIKPAPPFNVTV
TFSGQYNISWRSDYEDPAFYMLKGKLQYELQYRNRGDPWAVSPRRKLISVDSRSVSLLPLEFRK
HPLWRLWKKIWAVPSPERFFMPLYKGCSGDFKKWVGAPFTGSSLELGPWSPEVPSTLEVYSCHP
PRSPAKRLQLTELQEPAELVESDGVPKPSFWPTAQNSGGSAYSEERDRPYGLVSIDTVTVLDAEG
PCTWPCSCEDDGYPALDLDAGLEPSPGLEDPLLDAGTTVLSCGCVSAGSPGLGGPLGSLLDRLKP
PLADGEDWAGGLPWGGRSPGGVSESEAGSPLAGLDMDTFDSGFVGSDCSSPVECDFTSPGDEGP
PRSYLRQWVVIPPPLSSPGPQAS* (SEQ ID NO: 13)
>ENSG00000103522_ENST00000564089_ENSP00000456707_IL21R
MPRGWAAPLLLLLLQGGWGCPDLVCYTDYLQTVICILEMWNLHPSTLTLTWQDQYEELKDEAT
SCSLHRSAHNATHATYTCHMDVFHFMADDIFSVNITDQSGNYSQECGSFLLAESIKPAPPFNVTV
TFSGQYNISWRSDYEDPAFYMLKGKLQYELQYRNRGDPWAVSPRRKLISVDSRSVSLLPLEFRK
DSSYELQVRAGPMPGSSYQGTWSEWSDPVIFQTQSEELKEGWNPHLLLLLLLVIVFIPAFWSLKT
HPLWRLWKKIWAVPSPERFFMPLYKGCSGDFKKWVGAPFTGSSLELGPWSPEVPSTLEVYSCHP
PRSPAKRLQLTELQEPAELVESDGVPKPSFWPTAQNSGGSAYSEERDRPYGLVSIDTVTVLDAEG
PCTWPCSCEDDGYPALDLDAGLEPSPGLEDPLLDAGTTVLSCGCVSAGSPGLGGPLGSLLDRLKP
PLADGEDWAGGLPWGGRSPGGVSESEAGSPLAGLDMDTFDSGFVGSDCSSPVECDFTSPGDEGP
PRSYLRQWVVIPPPLSSPGPQAS* (SEQ ID NO: 14)]).
[0030] Said cytokine is preferably selected from the group of consisting of: IL32 (SEQ ID Nos: 19-30), IL7 (SEQ ID Nos: 168-174), EB13 (SEQ ID NO: 175), SECTMI (SEQ ID Nos: 349-356), CSF1 (SEQ ID Nos: 585-592) and LTA (SEQ ID Nos: 657-658).
[0031] Said epigenetic factor is preferably selected from the group of consisting of: TDRD3 (SEQ ID NO: 712-718), KAT2B (SEQ ID NO:719), FOXA1 (SEQ ID Nos: 720-721) and RCBTB1 (SEQ ID Nos: 722-723).
[0032] Said kinase phosphatase is preferably selected from the group of consisting of: GSK3B (SEQ ID Nos: 724-725), SSH1 (SEQ ID NOS:111-112), CDK6 (SEQ ID Nos: 726-727), MINPP1 (SEQ ID Nos:181-183), PTPRJ (SEQ ID Nos: 395-400), CALM3 (SEQ ID Nos: 728-734) and PTP4A3 (SEQ ID Nos: 593-598).
[0033] Said transcription factor is preferably selected from the group of consisting of: VDR (SEQ ID NO:204), ZNF334 (SEQ ID Nos: 736-741), CREB3L2 (SEQ ID Nos: 565-567), ETV7 (SEQ ID NO:31 or 32), SOX4 (SEQ ID NO:735), TWIST1 (SEQ ID Nos: 743-745), TP73 (SEQ ID Nos: 746-756), FOXP3, NFE2L3 (SEQ ID NO:76), ARNTL2 (SEQ ID Nos: 757-764), BATF (SEQ ID Nos: 765-766), PTTG1 (SEQ ID Nos: 767-770), HIVEP3 (SEQ ID Nos: 771-772), FOXA1 (SEQ ID Nos: 720-721), ZBTB38 (SEQ ID NO:561), FOXM1 (SEQ ID Nos: 773-778), TADA3 (SEQ ID Nos: 779-782), NFAT5 (SEQ ID NO:160, 783-791, 742).
[0034] In a preferred embodiment, the marker is MAGEH1 (SEQ ID NO: 708 or 709)
TABLE-US-00003
[MAGEH1_Entrez:28986_ENSG00000187601_ENST00000342972_ENSP00000343706
ATGCCTCGGGGACGAAAGAGTCGGCGCCGCCGTAATGCGAGAGCCGCAGAAGAGAACCGC
AACAATCGCAAAATCCAGGCCTCAGAGGCCTCCGAGACCCCTATGGCCGCCTCTGTGGTAGC
GAGCACCCCCGAAGACGACCTGAGCGGCCCCGAGGAAGACCCGAGCACTCCAGAGGAGGC
CTCTACCACCCCTGAAGAAGCCTCGAGCACTGCCCAAGCACAAAAGCCTTCAGTGCCCCGGA
GCAATTTTCAGGGCACCAAGAAAAGTCTCCTGATGTCTATATTAGCGCTCATCTTCATCATG
GGCAACAGCGCCAAGGAAGCTCTGGTCTGGAAAGTGCTGGGGAAGTTAGGAATGCAGCCTG
GACGTCAGCACAGCATCTTTGGAGATCCGAAGAAGATCGTCACAGAAGAGTTTGTGCGCAG
AGGGTACCTGATTTATAAACCGGTGCCCCGTAGCAGTCCGGTGGAGTATGAGTTCTTCTGGG
GGCCCCGAGCACACGTGGAATCGAGCAAACTGAAAGTCATGCATTTTGTGGCAAGGGTTCG
TAACCGATGCTCTAAAGACTGGCCTTGTAATTATGACTGGGATTCGGACGATGATGCAGAGG
TTGAGGCTATCCTCAATTCAGGTGCTAGGGGTTATTCCGCCCCTTAA (SEQ ID NO: 708)
MPRGRKSRRRRNARAAEENRNNRKIQASEASETPMAASVVASTPEDDLSGPEEDPSTPEEASTTP

EEASSTAQAQKPSVPRSNFQGTKKSLLMSILALIFIMGNSAKEALVWKVLGKLGMQPGRQHSIFG
DPKKIVTEEFVRRGYLIYKPVPRSSPVEYEFFWGPRAHVESSKLKVMHFVARVRNRCSKDWPCN
YDWDSDDDAEVEAILNSGARGYSAP* (SEQ ID NO: 709)].

[0035] In the present invention, the tumor is preferably a solid or liquid tumor. Preferably, the solid tumor is selected from the group consisting of: non-small cell lung cancer, colorectal cancer, breast cancer, gastric cancer.

[0036] In a preferred embodiment of the invention, the tumor is a metastasis, preferably a bone, a brain or a liver metastasis.

[0037] Preferably, the metastasis derives from colon rectal cancer or non-small-cell lung cancer.

[0038] Another object of the invention is the above defined molecule for use in a method for in vivo depleting tumor-infiltrating regulatory T cells in a subject or for use in a method to enhance tumor immunity in a subject.

[0039] Another object of the invention is a pharmaceutical composition comprising the molecule as defined above and at least one pharmaceutically acceptable carrier.

[0040] A further object of the invention is a pharmaceutical composition comprising the molecule as above defined, for use in the prevention and/or treatment of tumor or for use in a method for in vivo depleting tumor-infiltrating regulatory T cell in a subject or for use in a method to enhance tumor immunity in a subject.

[0041] The pharmaceutical composition according to the invention may further comprise a therapeutic agent, preferably the therapeutic agent in an anti-tumoral agent.

[0042] Another object of the invention is an in vitro method for diagnosing and/or assessing the risk of developing and/or prognosing and/or for monitoring the progression and/or for monitoring the efficacy of a therapeutic treatment and/or for the screening of a therapeutic treatment of a tumour in a subject comprising the steps of: [0043] a) detecting at least one of the marker as above defined in an isolated biological sample obtained from the subject and [0044] b) comparing with respect to a proper control.

[0045] Another object of the invention is an in vitro or ex-vivo method for diagnosing and/or assessing the risk of developing and/or prognosing and/or for monitoring the progression and/or for monitoring the efficacy of a therapeutic treatment and/or for the screening of a therapeutic treatment of a tumour in a subject as above defined, wherein the marker to be detected is at least one of the marker selected from the group consisting of: LAYN, MAGEH1 and CCR8.

[0046] Preferably the above method is for prognosing of colorectal cancer or non-small cell lung cancer in a subject and comprises the steps of: [0047] a) detecting at least one of the marker selected from the group consisting of: LAYN, MAGEH1 and CCR8 in an isolated biological sample obtained from the subject and [0048] b) comparing with respect to a proper control, [0049] wherein an amount of said at least one marker in the isolated biological sample obtained from the subject higher than the control amount indicates that the subject has a poor prognosis.

[0050] In the above method, preferably step a) comprises measuring the amount of the marker or of fragments thereof or of the polynucleotide coding for said protein (DNA or mRNA) or of fragments thereof in said isolated biological sample obtained from the subject and step b) comprises comparing the measured amount of step a) with a proper control amount.

[0051] Preferably, the in vitro method for monitoring the progression and/or for monitoring the efficacy of a therapeutic treatment of a tumour, as above defined, comprises the steps of: [0052] a) measuring the alteration of the amount or the alteration of the activity of the above markers or of fragments thereof or of the polynucleotide coding for said protein or fragments thereof in said isolated biological sample obtained from the subject and [0053] b) comparing the measured alteration of step a) with a proper control alteration.

[0054] Another object of the invention is a method for the treatment and/or prevention of tumor comprising administering to a subject the molecule as above defined.

[0055] A further object is a method for identifying a molecule acting as an anti-tumoral, comprising

the steps of: [0056] assaying candidate molecules for their binding specificity to the at least one marker as above defined; [0057] selecting molecules having a specific binding activity to the at least one marker as above defined; [0058] testing such specific binding molecules for their capacity of inhibiting proliferation and/or inducing an apoptotic response in a cell system, [0059] preferably by selectively depleting tumor-infiltrating regulatory T cell, more preferably by inducing antibody-dependent cell-mediated cytotoxicity (ADCC).
[0060] Preferably, the biological sample is a fluid, a cell or a tissue sample, more preferably said sample is plasma or serum.
[0061] The term “biological sample” encompasses a clinical sample, and also includes tissue obtained by surgical resection, tissue obtained by biopsy, cells in culture, cell supernatants, cell lysates, tissue samples, organs, bone marrow, blood, plasma, serum, and the like.
[0062] A “sample” in the context of the present teachings refers to any biological sample that is isolated from a subject. A sample can include, without limitation an aliquot of body fluid, whole blood, serum, plasma, solid tissue samples such as tissue biopsies, or tissue cultures or cells derived therefrom and the progeny thereof, synovial fluid, lymphatic fluid, ascites fluid, and interstitial or extracellular fluid. The term “sample” also encompasses the fluid in spaces between cells, including gingival crevicular fluid, bone marrow, cerebrospinal fluid (CSF), saliva, mucous, sputum, semen, sweat, urine, or any other bodily fluids. “Blood sample” can refer to whole blood or any fraction thereof, including serum and plasma. Samples can be obtained from a subject by means including but not limited to venipuncture, excretion, ejaculation, massage, biopsy, needle aspirate, lavage, scraping, surgical incision, or intervention or other means known in the art. The definition also includes samples that have been manipulated in any way after their procurement, such as by treatment with reagents; washed; or enrichment for certain cell populations, such as cancer cells or samples in which regulatory T cells, are isolated and then analyzed. The definition also includes sample that have been enriched for particular types of molecules, e.g., nucleic acids, polypeptides, etc.
[0063] Another object of the invention is a kit for carrying out the above methods, comprising
[0064] means to measure the amount or the activity of the above markers or of fragments thereof and/or means to measure the amount of the polynucleotide coding for said protein or of fragments thereof and optionally, [0065] control means.
[0066] Any combination of the above markers is comprised within the present invention.
[0067] Preferred combinations of markers are LAYN and MAGEH1; LAYN and CCR8; CCR8 and MAGEH1; LAYN, MAGEH1 and CCR8.
[0068] Preferably, the above polynucleotide is an RNAi inhibitor, preferably selected from the group consisting of: siRNA, miRNA, shRNA, stRNA, snRNA, and antisense nucleic acid, or a functional derivative thereof.
[0069] A comparative analysis of gene expression arrays from CD4+ T cells infiltrating NSCLC and CRC revealed Treg-specific expression of 328 markers as listed in Table IV Manipulation of Treg cells via these markers can therefore be used to enhance immunotherapy of cancer.
[0070] The expression “molecule able to modulate” and “modulator” are herein interchangeable.
[0071] By the term “modulator” it is meant a molecule that effects a change in the expression and/or function of at least one marker as above defined.
[0072] The change is relative to the normal or baseline level of expression and/or function in the absence of the modulator, but otherwise under similar conditions, and it may represent an increase (e.g. by using an inducer or activator) or a decrease (e.g. by using a suppressor or inhibitor) in the normal/baseline expression and/or function. In the context of the present invention, a “modulator” is a molecule which may suppress or inhibit the expression and/or function of at least one marker that is selectively deregulated in tumor-infiltrating regulatory T cell for use in the prevention and/or treatment of cancer.
[0073] By the term “suppressor or inhibitor” or a “molecule which (selectively) suppresses or

inhibits" it is meant a molecule that effects a change in the expression and/or function of the target.
[0074] In the context of the present invention, a "modulator" is a molecule which may induce or activate the expression and/or function of at least one marker that is selectively deregulated in tumor-infiltrating regulatory T cell for use in the prevention and/or treatment of cancer.
[0075] The change is relative to the normal or baseline level of expression and/or function in the absence of the modulator, but otherwise under similar conditions, and it may represent an increase (e.g. by using an inducer or activator) or a decrease (e.g. by using a suppressor or inhibitor) in the normal/baseline expression and/or function.
[0076] The suppression or inhibition of the expression and/or function of the target may be assessed by any means known to the skilled in the art. The assessment of the expression level or of the presence of the target is preferably performed using classical molecular biology techniques such as (real time Polymerase Chain Reaction) qPCR, microarrays, bead arrays, RNAse protection analysis or Northern blot analysis or cloning and sequencing.
[0077] The assessment of target function is preferably performed by in vitro suppression assay, whole transcriptome analysis, mass spectrometry analysis to identify proteins interacting with the target.
[0078] In the context of the present invention, the target (or the marker) may be the gene, the mRNA, the cDNA, or the encoded protein thereof, including fragments, derivatives, variants, isoforms, etc. Preferably, the marker is characterized by its Accession numbers (i.e. NCBI Entrez ID; Ensembl Gene accession number (ENSG), Ensembl transcript accession number (ENST) and Ensembl protein accession number (ENSP), retrievable in the public database EnsEMBL (http://www.ensembl.orq) and/or amino acid and nucleotide sequences, herein disclosed.
[0079] In the context of the present invention, the term "treat" (or "treated", "treatment", etc.) when referred to CD4+ T cell, means e.g. the exposure of the cell to an exogenous modulator as above defined. The overexpression may be obtained e.g. by infecting the cells with a viral vector expressing the molecule of the invention. The inhibition of marker expression may e.g. by obtained by transfection with polynucleotide, as e.g. with siRNAs.
[0080] The term "treat" may also mean that the cells are manipulated in order to overexpress or silence the marker. The overexpression or the silencing may be obtained e.g. by genetically modifying the cells.
[0081] Control means can be used to compare the amount or the increase of amount of the marker defined to a proper control. The proper control may be obtained for example, with reference to known standard, either from a normal subject or from normal population, or from T cells different from tumour infiltrating regulatory T cells or regulatory T cells. The means to measure the amount of at least one marker as above defined are preferably at least one antibody, functional analogous or derivatives thereof. Said antibody, functional analogous or derivatives thereof are specific for said marker.
[0082] In the context of the present invention, the antibody is preferably selected from the group consisting of an intact immunoglobulin, a Fv, a scFv (single chain Fv fragment), a Fab, a F(ab′)2, an "antibody-like" domain, an "antibody-mimetic domain", a single antibody domain (VH domain or VL domains), a multimeric antibody, recombinant or synthetic antigen-binding fragments, a peptide or a proteolytic fragment containing the epitope binding region. The terms "antibody" and "immunoglobulin" can be used interchangeably and are herein used in the broadest sense and encompass various antibodies and antibody mimetics structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), chimeric antibodies, nanobodies, antibody derivatives, antibody fragments, anticalins, DARPins, affibodies, affilins, affimers, affitins, alphabodies, avimers, fynomers, monobodies and other binding domains, so long as they exhibit the desired antigen-binding activity.
[0083] The term immunoglobulin also includes "conjugate" thereof. In the context of the present invention "conjugate" in relation to the antibody of the invention includes antibodies (or fragments

thereof) conjugated with a substance (a compound, etc.) having a therapeutic activity, e.g. anti-tumor activity and/or cell-killing activity or a cytotoxic agents such as various A chain toxins, ribosomes inactivating proteins, and ribonucleases; bispecific antibodies designed to induce cellular mechanisms for killing tumors (see, for example, U.S. Pat. Nos. 4,676,980 and 4,954,617). The conjugate may be formed by previously preparing each of the aforementioned antibody molecule and the aforementioned substance having anti-tumor activity and/or cell-killing activity, separately, and then combining them (immunoconjugate) or by ligating a protein toxin used as such a substance having anti-tumor activity and/or cell-killing activity to an antibody gene on a gene according to a genetic recombination technique, so as to allow it to express as a single protein (a fusion protein) (immunotoxin).

[0084] An “antibody fragment” refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds.

[0085] Examples of antibody fragments include but are not limited to Fv, Fab, Fab′, Fab′-SH, F(ab′)2; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv); and multispecific antibodies formed from antibody fragments. VH or VL Fvs are also called “Nanobodies”.

[0086] The term “antibody mimetics” refers to those organic compounds or binding domains that are not antibody derivatives but that can bind specifically an antigen like antibodies do.

[0087] They include anticalins, DARPins, affibodies, affilins, affimers, affitins, alphabodies, avimers, fynomers, monobodies and others.

[0088] The term “chimeric” antibody refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.

[0089] The terms “full length antibody,” “intact antibody,” and “whole antibody” are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region as defined herein.

[0090] In a preferred embodiment, the kit of the invention comprises: [0091] a solid phase adhered antibody specific for said compound; [0092] detection means of the ligand specific-marker complex.

[0093] Alternatively, the reagents can be provided as a kit comprising reagents in a suspension or suspendable form, e.g. reagents bound to beads suitable for flow cytometry, preferably magnetic beads coated with antibody capture. The instructions may comprise instructions for conducting an antibody-based flow cytometry assay.

[0094] Detection means are preferably means able to detect and/or measure the amount of the described markers, e.g. means able to detect the complex antigen-antibody, as enzyme conjugated secondary antibodies, luminescent substrates, magnetic beads coated with antibody capture, customized dried antibody cocktails and/or columns with size filter cartridges and/or combined with specific antibody filter (SAF).

[0095] In an embodiment, the method further comprises selecting a therapeutic regimen based on the analysis. In an embodiment, the method further comprises determining a treatment course for the subject based on the analysis. Other means may be e.g. specific primers and probes for RT PCR. The kits according to the invention can further comprise customary auxiliaries, such as buffers, carriers, markers, etc. and/or instructions for use.

[0096] In the context of the present invention the term “detecting” may be intended also as “measuring the amount” or “measuring the alteration”. In the case of a method or a kit for assessing the risk and/or diagnosing and/or prognosing of a tumour, the proper control may be a sample taken from a healthy patient or from a patient affected by another disorder or pathology, and the proper control amount or activity may be the amount or activity of the same protein or polynucleotide measured in a sample taken from a healthy patient or from a patient affected by another disorder or pathology.

[0097] In the case of a method or a kit for monitoring the progression of a tumour, the progress of

the cancer is monitored and the proper control may be a sample taken from the same subject at various times or from another patient, and the proper control amount or activity may by the amount or activity of the same protein or polynucleotide measured in a sample taken from the same subject at various times or from another patient.

[0098] In the case of a method or a kit for monitoring the efficacy of a therapeutic treatment, the proper control may by a sample taken from the same subject before initiation of the therapy or taken at various times during the course of the therapy and the proper control amount or activity may be the amount or activity of the same protein or polynucleotide measured in a sample taken from the same subject before initiation of the therapy or taken at various times during the course of the therapy.

[0099] In the case of a method or a kit for the screening of a therapeutic treatment, the proper control may be a sample taken from subjects without treatment and from subjects treated with a substance that is to be assayed or from subjects treated with a reference treatment and the proper control amount or activity may be the average of the amounts or activity of the same protein or polynucleotide measured in samples taken from subjects without treatment and from subjects treated with a substance that is to be assayed or from subjects treated with a reference treatment. In this case, if the amount or activity of MAGEH1 and/or LAYN and/or CCR8 or polynucleotides thereof in the isolated biological sample obtained from the subject is lower or equal than the control amount or activity, it may indicate that the tested substance is effective for the treatment of the tumour.

[0100] In the present invention, the expression “measuring the amount” can be intended as measuring the amount (or the activity) or concentration or level of the respective protein and/or mRNA thereof and/or DNA thereof, preferably semi-quantitative or quantitative.

[0101] Measurement of a protein can be performed directly or indirectly. Direct measurement refers to the amount or concentration measure of the marker, based on a signal obtained directly from the protein, and which is directly correlated with the number of protein molecules present in the sample. This signal—which can also be referred to as intensity signal—can be obtained, for example, by measuring an intensity value of a chemical or physical property of the marker. Indirect measurements include the measurement obtained from a secondary component (e.g., a different component from the gene expression product) and a biological measurement system (e.g. the measurement of cellular responses, ligands, “tags” or enzymatic reaction products).

[0102] The term “amount”, as used in the description refers but is not limited to the absolute or relative amount of proteins and/or mRNA thereof and/or DNA thereof, and any other value or parameter associated with the same or which may result from these. Such values or parameters comprise intensity values of the signal obtained from either physical or chemical properties of the protein, obtained by direct measurement, for example, intensity values in an immunoassay, mass spectroscopy or a nuclear magnetic resonance.

[0103] Additionally, these values or parameters include those obtained by indirect measurement, for example, any of the measurement systems described herein. Methods of measuring mRNA and DNA in samples are known in the art. To measure nucleic acid levels, the cells in a test sample can be lysed, and the levels of mRNA in the lysates or in RNA purified or semi-purified from lysates can be measured by any variety of methods familiar to those in the art. Such methods include hybridization assays using detectably labeled DNA or RNA probes (i.e., Northern blotting) or quantitative or semi-quantitative RT-PCR methodologies using appropriate oligonucleotide primers. Alternatively, quantitative or semi-quantitative in situ hybridization assays can be carried out using, for example, tissue sections, or unlysed cell suspensions, and detectably labeled (e.g., fluorescent, or enzyme-labeled) DNA or RNA probes. Additional methods for quantifying mRNA include RNA protection assay (RPA), cDNA and oligonucleotide microarrays, representation difference analysis (RDA), differential display, EST sequence analysis, and serial analysis of gene expression (SAGE).

[0104] If by comparing the measured amount or activity of the above markers or of the

polynucleotide coding for said protein with the amount or activity obtained from a control sample, the amount or the activity of said marker in the sample isolated from the subject corresponds to a higher value, the subject may present cancer or go towards an aggravation of said disease.
[0105] If by comparing the measured amount or activity of the above markers or of the polynucleotide coding for said protein with the amount or the activity obtained from a control sample, the amount or the activity of said marker in the sample isolated from the subject corresponds to a similar or lower value, the subject may be not affected by cancer or go toward an amelioration of cancer, respectively.
[0106] Alternatively, the expression “detecting” or “measuring the amount” is intended as measuring the alteration of the molecule. Said alteration can reflect an increase or a decrease in the amount or activity of the molecules as above defined. An increase of the protein or of the activity of the marker or of the polynucleotide coding for said marker can be correlated to an aggravation of cancer. A decrease of the protein or of the activity of said marker or of the polynucleotide coding for said protein can be correlated to an amelioration of cancer or to recovery of the subject.
[0107] The expression “marker” is intended to include also the corresponding protein encoded from said marker orthologous or homologous genes, functional mutants, functional derivatives, functional fragments or analogues, isoforms, splice variants thereof.
[0108] When the expression “marker” is referred to genes, it is intended to include also the corresponding orthologous or homologous genes, functional mutants, functional derivatives, functional fragments or analogues, isoforms thereof.
[0109] As used herein “fragments” refers to polynucleotides having preferably a length of at least 1000 nucleotides, 1100 nucleotide, 1200 nucleotides, 1300 nucleotides, 1400 nucleotides, 1500 nucleotides.
[0110] As used herein “fragments” refers to polypeptides having preferably a length of at least amino acids, more preferably at least 15, at least 17 amino acids or at least 20 amino acids, even more preferably at least 25 amino acids or at least 37 or 40 amino acids, and more preferably of at least 50, or 100, or 150 or 200 or 250 or 300 or 350 or 400 or 450 or 500 amino acids.
[0111] The term “polynucleotide” also refers to modified polynucleotides.
[0112] As used herein, the term “vector” refers to an expression vector, and may be for example in the form of a plasmid, a viral particle, a phage, etc. Such vectors may include bacterial plasmids, phage DNA, baculovirus, yeast plasmids, vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, lentivirus, fowl pox virus, and pseudorabies. Large numbers of suitable vectors are known to those of skill in the art and are commercially available.
[0113] The polynucleotide sequence, preferably the DNA sequence in the vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As representative examples of such promoters, one can mention prokaryotic or eukaryotic promoters such as CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-1. The expression vector may also contain a ribosome binding site for translation initiation and a transcription vector.
[0114] The vector may also include appropriate sequences for amplifying expression. In addition, the vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydro folate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in *E. coli.*
[0115] As used herein, the term “host cell genetically engineered” relates to host cells which have been transduced, transformed or transfected with the polynucleotide or with the vector described previously. As representative examples of appropriate host cells, one can cite bacterial cells, such as *E. coli*, *Streptomyces*, *Salmonella typhimurium*, fungal cells such as yeast, insect cells such as Sf9, animal cells such as CHO or COS, plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein. Preferably, said host cell is an animal cell, and most preferably a human cell.

[0116] The introduction of the polynucleotide or of the vector described previously into the host cell can be effected by method well known from one of skill in the art such as calcium phosphate transfection, DEAE-Dextran mediated transfection, electroporation, lipofection, microinjection, viral infection, thermal shock, transformation after chemical permeabilisation of the membrane or cell fusion.
[0117] The polynucleotide may be a vector such as for example a viral vector.
[0118] The polynucleotides as above defined can be introduced into the body of the subject to be treated as a nucleic acid within a vector which replicates into the host cells and produces the polynucleotides or the proteins.
[0119] Suitable administration routes of the pharmaceutical composition of the invention include, but are not limited to, oral, rectal, transmucosal, intestinal, enteral, topical, suppository, through inhalation, intrathecal, intraventricular, intraperitoneal, intranasal, intraocular and parenteral (e.g., intravenous, intramuscular, intramedullary, and subcutaneous). An additional suitable administration route includes chemoembolization. Other suitable administration methods include injection, viral transfer, use of liposomes, e.g. cationic liposomes, oral intake and/or dermal application.
[0120] In certain embodiments, a pharmaceutical composition of the present invention is administered in the form of a dosage unit (e.g., tablet, capsule, bolus, etc.).
[0121] For pharmaceutical applications, the composition may be in the form of a solution, e.g. an injectable solution, emulsion, suspension or the like. The carrier may be any suitable pharmaceutical carrier. Preferably, a carrier is used which is capable of increasing the efficacy of the RNA molecules to enter the target cells. Suitable examples of such carriers are liposomes.
[0122] The modulator as above defined is administered in a pharmaceutically effective dosage, which in the case of polynucleotides may be in the range of 0.001 pg/kg body weight to 10 mg/kg body weight depending on the route of administration and the type or severity of the disease.
[0123] The term “pharmaceutical composition” refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered. In the present invention the term “effective amount” shall mean an amount which achieves a desired effect or therapeutic effect as such effect is understood by those of ordinary skill in the art. In the present invention, the antibody may be administered simultaneously or sequentially with another therapeutic treatment, that may be a chemotherapy or radiotherapy. The invention provides formulations comprising a therapeutically effective amount of an antibody as disclosed herein, a buffer maintaining the pH in the range from about 4.5 to about 8.5, and, optionally, a surfactant. The formulations are typically for an antibody as disclosed herein, recombinant or synthetic antigen-binding fragments thereof of the invention as active principle concentration from about 0.1 mg/ml to about 100 mg/ml. In certain embodiments, the antibody, recombinant or synthetic antigen-binding fragments thereof concentration is from about 0.1 mg/ml to 1 mg/ml; preferably from 1 mg/ml to 10 mg/ml, preferably from 10 to 100 mg/ml.
[0124] Therapeutic formulations of the antibody/antibodies can be prepared by mixing the antibody having the desired degree of purity with optional physiologically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed., 1980), in the form of lyophilized formulations or aqueous solutions.
[0125] Pharmaceutical compositions containing the antibody of the present invention may be manufactured by processes well known in the art, e.g., using a variety of well-known mixing, dissolving, granulating, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes. Proper formulation is dependent upon the route of administration chosen. Parenteral routes are preferred in many aspects of the invention.
[0126] For injection, including, without limitation, intravenous, intramusclular and subcutaneous injection, the compounds of the invention may be formulated in aqueous solutions, preferably in

physiologically compatible buffers such as physiological saline buffer or polar solvents including, without limitation, a pyrrolidone or dimethylsulfoxide.

[0127] Formulations for injection may be presented in unit dosage form, e.g. in ampoules or in multi-dose containers. Useful compositions include, without limitation, suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain adjuncts such as suspending, stabilizing and/or dispersing agents. Pharmaceutical compositions for parenteral administration include aqueous solutions of a water soluble form, such as, without limitation, a salt of the active compound. Additionally, suspensions of the active compounds may be prepared in a lipophilic vehicle. Suitable lipophilic vehicles include fatty oils such as sesame oil, synthetic fatty acid esters such as ethyl oleate and triglycerides, or materials such as liposomes. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers and/or agents that increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water, before use. For administration by inhalation, the antibody of the present invention can conveniently be delivered in the form of an aerosol spray using a pressurized pack or a nebulizer and a suitable propellant. The antibody may also be formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides. In addition to the formulations described previously, the antibody may also be formulated as depot preparations. Such long acting formulations may be administered by implantation (for example, subcutaneously or intramuscularly) or by intramuscular injection. The compounds of this invention may be formulated for this route of administration with suitable polymeric or hydrophobic materials (for instance, in an emulsion with a pharmacologically acceptable oil), with ion exchange resins, or as a sparingly soluble derivative such as, without limitation, a sparingly soluble salt. Additionally, the antibody may be delivered using a sustained-release system, such as semi-permeable matrices of solid hydrophobic polymers containing the therapeutic agent. Other delivery systems such as liposomes and emulsions can also be used.

[0128] A therapeutically effective amount refers to an amount of compound effective to prevent, alleviate or ameliorate cancer or cancer recurrence symptoms. Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the disclosure herein. For any antibody used in the invention, the therapeutically effective amount can be estimated initially from in vitro assays. Then, the dosage can be formulated for use in animal models so as to achieve a circulating concentration range that includes the effective dosage. Such information can then be used to more accurately determine dosages useful in patients. The amount of the composition that is administered will depend upon the parent molecule included therein.

[0129] Generally, the amount used in the treatment methods is that amount which effectively achieves the desired therapeutic result in mammals. Naturally, the dosages of the various compounds can vary somewhat depending upon the compound, rate of in vivo hydrolysis, etc. In addition, the dosage, of course, can vary depending upon the dosage form and route of administration. The range set forth above is illustrative and those skilled in the art will determine the optimal dosing of the compound selected based on clinical experience and the treatment indication. Moreover, the exact formulation, route of administration and dosage can be selected by the individual physician in view of the patient's condition and of the most effective route of administration (e.g., intravenous, subcutaneous, intradermal). Additionally, toxicity and therapeutic efficacy of the antibody and other therapeutic agent described herein can be determined by standard pharmaceutical procedures in cell cultures or experimental animals using methods well-known in the art.

[0130] It is contemplated that the treatment will be given for one or more cycles until the desired clinical and biological result is obtained. The exact amount, frequency and period of administration

of the compound of the present invention will vary, of course, depending upon the sex, age and medical condition of the patient as well as the severity and type of the disease as determined by the attending clinician.
[0131] The modulator of the present invention may comprise a single type of modulator or a plurality of different modulators.
[0132] The function of a regulatory T-cell may be inhibited by inhibiting markers activity and/or expression or by decreasing the number of cells positive for such markers in a T-cell population (for example by binding at least one of the above marker and inducing antibody-dependent cell-mediated cytotoxicity (ADCC)). Inhibiting the function of regulatory T-cells in an organism may be used to enhance the immune T-cell response in those circumstances where such a response is desirable, such as in a patient suffering from cancer.
[0133] When treating a cancer patient with an inhibitory agent that binds to marker protein or mRNA, one may optionally co-administer an anti-tumor vaccine or therapy. Such vaccines may be directed to isolated antigens or to groups of antigens or to whole tumor cells. It may be desirable to administer the inhibitory agent with chemotherapeutic agents or together with radiotherapy.
[0134] Treatment with multiple agents need not be done using a mixture of agents but may be done using separate pharmaceutical preparations. The preparations need not be delivered at the same exact time, but may be coordinated to be delivered to a patient during the same period of treatment, i.e. within a week or a month or each other.
[0135] Thus a composition comprising two active ingredients may be constituted in the body of the patient. Any suitable anti-tumor treatment can be coordinated with the treatments of the present invention targeted to the markers. Similarly, if treating patients with infections, other anti-infection agents can be coordinated with the treatment of the present invention targeted to the markers. Such agents may be small molecule drugs, vaccines, antibodies, etc.
[0136] The number of marker+ cells in a T-cell population can be modified by using an antibody or other agent that selectively binds to the marker. marker+ cells represent an enriched population of regulatory T-cells that can be introduced back into the original source of the T-cells or into another compatible host to enhance regulatory T-cell function. Alternatively, the marker-cells represent a population of T-cells deficient in regulatory T-cell activity that can be reintroduced into the original source of the T-cells or another compatible host to inhibit or reduce regulatory T-cell function while retaining general T-cell activity.
[0137] Any desired means for either increasing or decreasing (modulating) marker activity can be used in the methods of the invention. This includes directly modulating the function of marker protein, modulating marker signal transduction, and modulating expression of marker in T-cells by modulating either transcription or translation or both. Those means which selectively modulate marker activity are preferred over nonselective modulators.
[0138] Also, those inhibitory means which create a transient marker deficiency in a population of T-cells which then return to normal levels of marker activity may be preferred for treating a temporary T-cell deficiency. The transiently deficient T-cells may be used to reconstitute a diminished T-cell population with T-cells that will be genetically normal with respect to the marker. Modulation of marker activity can be performed on cells in vitro or in whole animals, in vivo. Cells which are treated in vitro can be administered to a patient, either the original source of the cells or an unrelated individual.
[0139] To inhibit the function of the marker (antagonist), marker antibodies or small molecule inhibitors can be used. Antibodies or antibody fragments that are useful for this purpose will be those that can bind to the marker and block its ability to function. Such antibodies may be polyclonal antibodies, monoclonal antibodies, chimeric antibodies, humanized antibodies, single-chain antibodies, soluble MHC class II molecules, antibody fragments, etc.
[0140] Antibodies generated against marker polypeptides can be obtained by direct injection of the marker polypeptides into an animal or by administering marker polypeptides to an animal,

preferably a nonhuman. The antibody so obtained will then bind the marker polypeptides itself. In this manner, even a sequence encoding only a fragment of the marker polypeptide can be used to generate antibodies binding the whole native marker polypeptide.
[0141] For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein, 1975, Nature, 256: 495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4: 72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole, et al; 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Techniques described for the production of single chain antibodies (U.S. Pat. No. 4,946,778) can be readily used to produce single chain antibodies to marker polypeptides. Also, transgenic mice may be used to express humanized antibodies to immunogenic marker polypeptides. To enhance or activate the function of the marker, any agent which increases the level of the marker or the activity of existing marker in the T-cell may be used. Such agents may be identified using the screening assays described below. Expression vectors encoding the marker can also be administered to increase the gene dosage. The expression vectors can be plasmid vectors or viral vectors, as are known in the art. Any vector can be chosen by the skilled in the art for particularly desirable properties. In the context of the present invention, the term “polynucleotide” includes DNA molecules (e.g., cDNA or genomic DNA) and RNA molecules (e.g., mRNA, siRNA, shRNA) and analogues of the DNA or RNA generated using nucleotide analogues. The polynucleotide may be single-stranded or double-stranded. The polynucleotide may be synthesized using oligonucleotide analogues or derivatives (e.g., inosine or phosphorothioate nucleotides).
[0142] The RNAi inhibitors as above defined are preferably capable of hybridizing to all or part of specific target sequence. Therefore, RNAi inhibitors may be fully or partly complementary to all of or part of the target sequence
[0143] The RNAi inhibitors may hybridize to the specified target sequence under conditions of medium to high stringency.
[0144] An RNAi inhibitors may be defined with reference to a specific sequence identity to the reverse complement of the sequence to which it is intended to target. The antisense sequences will typically have at least about 75%, preferably at least about 80%, at least about 85%, at least about 90%, at least about 95% or at least about 99% sequence identity with the reverse complements of their target sequences.
[0145] The term polynucleotide and polypeptide also includes derivatives and functional fragments thereof.
[0146] In the context of the present invention, the at least one gene or marker as above defined is preferably characterized by at least one of the sequence identified by its Ensembl Gene ID or NCBI Accession Numbers, as disclosed in Tables VIII or VI, or by at least one of the SEQ ID No. 1-709.
[0147] The term gene herein also includes corresponding orthologous or homologous genes, isoforms, variants, allelic variants, functional derivatives, functional fragments thereof.
[0148] The expression “protein” is intended to include also the corresponding protein encoded from a corresponding orthologous or homologous genes, functional mutants, functional derivatives, functional fragments or analogues, isoforms thereof.
[0149] The term “analogue” as used herein referring to a protein means a modified peptide wherein one or more amino acid residues of the peptide have been substituted by other amino acid residues and/or wherein one or more amino acid residues have been deleted from the peptide and/or wherein one or more amino acid residues have been deleted from the peptide and or wherein one or more amino acid residues have been added to the peptide. Such addition or deletion of amino acid residues can take place at the N-terminal of the peptide and/or at the C-terminal of the peptide.
[0150] A “derivative” may be a nucleic acid molecule, as a DNA molecule, coding the polynucleotide as above defined, or a nucleic acid molecule comprising the polynucleotide as above

defined, or a polynucleotide of complementary sequence. In the context of the present invention the term “derivatives” also refers to longer or shorter polynucleotides and/or polypeptides having e.g. a percentage of identity of at least 41%, 50%, 60%, 65%, 70% or 75%, more preferably of at least 85%, as an example of at least 90%, and even more preferably of at least 95% or 100% with the sequences herein mentioned or with their complementary sequence or with their DNA or RNA corresponding sequence. The term “derivatives” and the term “polynucleotide” also include modified synthetic oligonucleotides. The modified synthetic oligonucleotide are preferably LNA (Locked Nucleic Acid), phosphoro-thiolated oligos or methylated oligos, morpholinos, 2′-O-methyl, 2′-O-methoxyethyl oligonucleotides and cholesterol-conjugated 2′-O-methyl modified oligonucleotides (antagomirs).

[0151] The term “derivative” may also include nucleotide analogues, i.e. a naturally occurring ribonucleotide or deoxyribonucleotide substituted by a non-naturally occurring nucleotide.

[0152] The term “derivatives” also includes nucleic acids or polypeptides that may be generated by mutating one or more nucleotide or amino acid in their sequences, equivalents or precursor sequences. The term “derivatives” also includes at least one functional fragment of the polynucleotide.

[0153] In the context of the present invention “functional” is intended for example as “maintaining their activity”.

[0154] In the context of the present invention, the vector as above defined is preferably selected from the group consisting of: plasmids, viral vectors and phages, more preferably the viral vector is a lentiviral vector.

[0155] In the context of the present invention, the host cell as above defined is preferably selected from the group consisting of: bacterial cells, fungal cells, insect cells, animal cells, plant cells, preferably being an animal cell.

[0156] Compositions comprising a mixture of antibodies which specifically bind to the marker(s); and an anti-cancer vaccine can be made in vitro. Preferably the composition is made under conditions which render it suitable for use as a pharmaceutical composition.

[0157] Pharmaceutical compositions may be sterile and pyrogen-free. The components of the composition can also be administered separately to a patient within a period of time such that they are both within the patient's body at the same time. Such a time-separated administration leads to formation of the mixture of antibodies and vaccine within the patient's body. If the antibody and vaccine are to be administered in a time-separated fashion, they may be supplied together in a kit. Within the kit the components may be separately packaged or contained. Other components such as excipients, carriers, other immune modulators or adjuvants, instructions for administration of the antibody and the vaccine, and injection devices can be supplied in the kit as well. Instructions can be in a written, video, or audio form, can be contained on paper, an electronic medium, or even as a reference to another source, such as a website or reference manual.

[0158] Anti-marker antibodies of the invention can be used to increase the magnitude of anti-cancer response of the cancer patient to the anti-cancer vaccine or anti-cancer therapy.

[0159] It can also be used to increase the number of responders in a population of cancer patients. Thus the antibodies can be used to overcome immune suppression found in patients refractory to anti-cancer vaccines or treatment. The anti-cancer vaccines can be any that are known in the art, including, but not limited to whole tumor cell vaccines, isolated tumor antigens or polypeptides comprising one or more epitopes of tumor antigens.

[0160] Expression of marker in T-cells can be modulated at the transcriptional or translational level. Agents which are capable of such modulation can be identified using the screening assays described below.

[0161] Translation of marker mRNA can be inhibited by using ribozymes, antisense molecules, small interference RNA (siRNA; See Elbashir, S. M. et al., “Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells”, Nature 411: 494-498 (2001)) or small

molecule inhibitors of this process which target marker mRNA.
[0162] Antisense technology can be used to control gene expression through triple-helix formation or antisense DNA or RNA, both of which methods are based on binding of a polynucleotide to DNA or RNA. For example, the 5′coding portion of the polynucleotide sequence, which codes for the mature polypeptides of the present invention, is used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription (triple helix—see Lee et al., Nucl. Acids Res., 6: 3073 (1979); Cooney et al, Science, 241: 456 (1988); and Dervan et al., Science, 251: 1360 (1991)), thereby preventing transcription and the production of the marker. The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into the marker polypeptide (Antisense—Okano, J. Neurochem., 56: 560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, Fla. (1988)). The oligonucleotides described above can also be delivered to cells by antisense expression constructs such that the antisense RNA or DNA may be expressed in vivo to inhibit production of the marker. Such constructs are well known in the art. Antisense constructs, antisense oligonucleotides, RNA interference constructs or siRNA duplex RNA molecules can be used to interfere with expression of the marker.
[0163] Typically, at least 15, 17, 19, or 21 nucleotides of the complement of marker mRNA sequence are sufficient for an antisense molecule. Typically at least 19, 21, 22, or 23 nucleotides of marker are sufficient for an RNA interference molecule. Preferably an RNA interference molecule will have a 2 nucleotide 3′ overhang. If the RNA interference molecule is expressed in a cell from a construct, for example from a hairpin molecule or from an inverted repeat of the desired marker sequence, then the endogenous cellular machinery will create the overhangs. siRNA molecules can be prepared by chemical synthesis, in vitro transcription, or digestion of long dsRNA by Rnase III or Dicer. These can be introduced into cells by transfection, electroporation, or other methods known in the art. (See Hannon, G J, 2002, RNA Interference, Nature 418:244-251; Bernstein E et al., 2002, The rest is silence. RNA 7:1509-1521; Hutvagner G et aL9 RNAi: Nature harbors a double-strand. Curr. Opin. Genetics & Development 12: 225-232, 2002, A system for stable expression of short interfering RNAs in mammalian cells. Science 296: 550-553; Lee N S, Dohjima T, Bauer G, Li H, Li M-J, Ehsani A, Salvaterra P, and Rossi J. (2002). Expression of small interfering RNAs targeted against HIV-1 rev transcripts in human cells. Nature Biotechnol. 20: 500-505; Miyagishi M, and Taira K. (2002). U6-promoter-driven siRNAs with four uridine 3′ overhangs efficiently suppress targeted gene expression in mammalian cells. Nature Biotechnol. 20: 497-500; Paddison P J, Caudy A A, Bernstein E, Hannon G J, and Conklin D S. (2002). Short hairpin RNAs (shRNAs) induce sequence-specific silencing in mammalian cells. Genes & Dev. 16: 948-958; Paul C P, Good P D, Winer I, and Engelke D R. (2002). Effective expression of small interfering RNA in human cells. Nature Biotechnol. 20: 505-508; Sui G, Soohoo C, Affar E-B, Gay F, Shi Y, Forester W C, and Shi Y. (2002). A DNA vector-based RNAi technology to suppress gene expression in mammalian cells. Proc. Natl. Acad. Sci. USA 99 (6): 5515-5520; Yu J-Y, DeRuiter S L, and Turner D L. (2002). RNA interference by expression of short-interfering RNAs and hairpin RNAs in mammalian cells. Proc. Natl. Acad. Sci. USA 99 (9): 6047-6052).
[0164] In addition to known modulators, additional modulators of markers activity that are useful in the methods of the invention can be identified using two-hybrid screens, conventional biochemical approaches, and cell-based screening techniques, such as screening candidate molecules for an ability to bind to marker or screening for compounds which inhibit marker activity in cell culture.
[0165] This provides a simple in vitro assay system to screen for marker activity modulators. The method may identify agents that directly interact with and modulate the marker, as well as agents that indirectly modulate marker activity by affecting a step in the marker signal transduction pathway.
[0166] Cell-based assays employing cells which express the marker can employ cells which are

isolated from mammals and which naturally express the marker. Alternatively, cells which have been genetically engineered to express the marker can be used. Preferably the genetically engineered cells are T-cells.

[0167] Agents which modulate the marker activity by modulating the markergene expression can be identified in cell based screening assays by measuring amounts of the marker protein in the cells in the presence and absence of candidate agents. The marker protein can be detected and measured, for example, by flow cytometry using anti-marker specific monoclonal antibodies. Marker mRNA can also be detected and measured using techniques known in the art, including but not limited to Northern blot, RT-PCR, and array hybridization.

[0168] In accordance with the teachings of the invention, marker inhibitors may be administered to an organism to increase the number of T-cells in the organism. This method may be useful for treating organisms suffering from conditions resulting in a low T-cell population.

[0169] Such conditions include disorders involving unwanted cellular invasion or growth, such as tumor growth or cancer. Marker inhibitors may also be useful when administered in combination with conventional therapeutics to treat T-cell proliferation sensitive disorders.

[0170] For instance, a tumor, which is a T-cell proliferation sensitive disorder, is conventionally treated with a chemotherapeutic agent which functions by killing rapidly dividing cells. The marker inhibitors of the invention when administered in conjunction with a chemotherapeutic agent enhance the tumoricidal effect of the chemotherapeutic agent by stimulating T-cell proliferation to enhance the immunological rejection of the tumor cells.

[0171] In accordance with the teachings of the invention, marker activators (agonists) or expression enhancers may be administered to an organism to decrease the number of T-cells, in particular tumor-infiltrating regulatory T cells, in the organism and thereby decrease deleterious T-cell activity. The methods of the invention may be applied to any organism which contains T-cells that express the marker. This includes, but is not limited to, any mammal and particularly includes humans and mice.

[0172] When methods of the invention are carried out in vivo, the effective amount of the marker modulator used will vary with the particular modulator being used, the particular condition being treated, the age and physical condition of the subject being treated, the severity of the condition, the duration of the treatment, the nature of the concurrent therapy (if any), the specific route of administration and similar factors within the knowledge and expertise of the health practitioner. For example, an effective amount can depend upon the degree to which an individual has abnormally depressed levels of T cells.

[0173] When administered, the pharmaceutical preparations of the invention are applied in pharmaceutically-acceptable amounts and in pharmaceutically-acceptably compositions.

[0174] Such preparations may routinely contain salt, buffering agents, preservatives, compatible carriers, and optionally other therapeutic agents. When used in medicine, the salts should be pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare pharmaceutically-acceptable salts thereof and are not excluded from the scope of the invention. Such pharmacologically and pharmaceutically-acceptable salts include, but are not limited to those prepared from the following acids: hydrochloric, hydrobromic, sulfuric, nitric, phosphoric, maleic, acetic, salicylic, citric, formic, malonic, succinic, and the like. Also, pharmaceutically-acceptable salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts. Marker modulators may be combined, optionally, with a pharmaceutically-acceptable carrier. The term “pharmaceutically-acceptable carrier” as used herein means one or more compatible solid or liquid filler, diluents or encapsulating substances which are suitable for administration into a human. The term “carrier” denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. The components of the pharmaceutical compositions also are capable of being co-mingled with the molecules of the present invention, and with each other, in a manner such that

there is no interaction which would substantially impair the desired pharmaceutical efficacy.
[0175] The pharmaceutical compositions may contain suitable buffering agents, including: acetic acid in a salt; citric acid in a salt; boric acid in a salt; and phosphoric acid in a salt. The pharmaceutical compositions also may contain, optionally, suitable preservatives, such as: benzalkonium chloride; chlorobutanol; parabens and thimerosal.
[0176] Compositions suitable for parenteral administration conveniently comprise a sterile aqueous preparation of the anti-inflammatory agent, which is preferably isotonic with the blood of the recipient. This aqueous preparation may be formulated according to known methods using suitable dispersing or wetting agents and suspending agents.
[0177] The sterile injectable preparation also may be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in butane diol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or di-glycerides. In addition, fatty acids such as oleic acid may be used in the preparation of injectables.
[0178] Carrier formulation suitable for oral, subcutaneous, intravenous, intramuscular, etc. administrations can be found in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa.
[0179] A variety of administration routes are available. The particular mode selected will depend, of course, upon the particular drug selected, the severity of the condition being treated and the dosage required for therapeutic efficacy. The methods of the invention, generally speaking, may be practiced using any mode of administration that is medically acceptable, meaning any mode that produces effective levels of the active compounds without causing clinically unacceptable adverse effects. Such modes of administration include oral, rectal, topical, nasal, interdermal, or parenteral routes.
[0180] The term “parenteral” includes subcutaneous, intravenous, intramuscular, or infusion.
[0181] Intravenous or intramuscular routes are not particularly suitable for long-term therapy and prophylaxis. They could, however, be preferred in emergency situations. Oral administration will be preferred because of the convenience to the patient as well as the dosing schedule. The pharmaceutical compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods well-known in the art of pharmacy. All methods include the step of bringing the active agent into association with a carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing the active agent into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product. Compositions suitable for oral administration may be presented as discrete units, such as capsules, tablets, lozenges, each containing a predetermined amount of the active agent. Other compositions include suspensions in aqueous liquids or non-aqueous liquids such as a syrup, elixir or an emulsion.
[0182] Other delivery systems can include time-release, delayed release or sustained release delivery systems. Such systems can avoid repeated administrations of the active agent, increasing convenience to the subject and the physician. Many types of release delivery systems are available and known to those of ordinary skill in the art. They include polymer base systems such as poly (lactide-glycolide), copolyoxalates, polycaprolactones, polyesteramides, polyorthoesters, polyhydroxybutyric acid, and polyanhydrides.
[0183] Microcapsules of the foregoing polymers containing drugs are described in, for example, U.S. Pat. No. 5,075,109. Delivery systems also include non-polymer systems that are: lipids including sterols such as cholesterol, cholesterol esters and fatty acids or neutral fats such as mono-di- and tri-glycerides; hydrogel release systems; sylastic systems; peptide based systems; wax coatings; compressed tablets using conventional binders and excipients; partially fused implants;

and the like. Specific examples include, but are not limited to: (a) erosional systems in which the anti-inflammatory agent is contained in a form within a matrix such as those described in U.S. Pat. Nos. 4,452,775, 4,667,014, 4,748,034 and 5,239,660 and (b) diffusional systems in which an active component permeates at a controlled rate from a polymer such as described in U.S. Pat. Nos. 3,832,253, and 3,854,480. In addition, pump-based hardware delivery systems can be used, some of which are adapted for implantation.

[0184] Use of a long-term sustained release implant may be particularly suitable for treatment of chronic conditions. Long-term release, are used herein, means that the implant is constructed and arranged to deliver therapeutic levels of the active ingredient for at least 30 days, and preferably 60 days. Long-term sustained release implants are well-known to those of ordinary skill in the art and include some of the release systems described above. While the invention has been described with respect to specific examples including presently preferred modes of carrying out the invention, those skilled in the art will appreciate that there are numerous variations and permutations of the above described systems and techniques that fall within the spirit and scope of the invention as set forth in the appended claims.

Description

[0185] The invention will be illustrated by means of non-limiting examples in reference to the following figures.

[0186] FIG. **1**. Purification, functional characterization and expression of immune checkpoints in tumor infiltrating cells.

[0187] (A) Representation of the sorting strategy of Treg cells infiltrating tumor or normal tissue.

[0188] (B) Representative flow cytometry plots showing suppressive activity of Treg cells isolated from tumor (NSCLC or CRC), normal lung and blood of the same patient. 4×10.sup.5 carboxyfluorescein diacetate succinimidyl ester (CFSE)-labeled CD4+ naïve T cells from healthy donors were cocultured with an equal number of Treg cells for 4 days with a CD3-specific mAb and CD1c+CD11c+ dendritic cells. Percentage of proliferating cells are indicated. Data are representative of three independent experiments.

[0189] (C) Z-score normalized RNA-seq expression values of immunecheckpoints genes are represented as a heatmap. Cell populations are reported in the upper part of the graph, while gene names have been assigned to heatmap rows. Hierarchical clustering results are shown as a dendrogram drawn on the left side of the matrix. Colon tissues are indicated as C, lung tissues as L and peripheral blood as B. See also FIG. **6**.

[0190] FIG. **2**. Differential expression analysis identifies co-regulated genes in tumor infiltrating Treg cells

[0191] Z-score normalized expression values of genes that are preferentially expressed in tumor-infiltrating Tregs (Wilcoxon Mann Whitney test $p<2.2\times10-16$) over the listed cell subsets are represented as boxed plots. Colon tissues are indicated as C, lung tissues as L and peripheral blood as B.

[0192] FIG. **3**. Single cell analysis of tumor infiltrating Treg cells

[0193] (A) Schematic representation of the experimental workflow. Experiments were performed on Treg cells infiltrating CRC, NSCLC, or isolated from peripheral blood of healthy donors (PB); five samples were collected for each tissue.

[0194] (B) Percentage of co-expression of signature genes with FOXP3 and IL2RA is depicted.

[0195] (C) Expression levels of the signature genes classified by the percentage of co-expression are represented as box plot.

[0196] (D) Expression distribution (violin plots) in Treg cells infiltrating CRC, NSCLC or PB. Plots representing the ontology classes of receptors, signaling and enzymatic activity, cytokine activity and transcription factors are shown (Wilcoxon Mann Whitney test $p<0.05$). Gray scale gradient

indicates the percentage of cells expressing each gene in Treg cells isolated from the three compartments.
[0197] (E) Gene expression analysis of tumor Treg signature genes in different tumor types. Expression values are expressed as log 2 (2{circumflex over ( )}−DCt).
[0198] FIG. **4**. Expression of tumor-infiltrating Treg cells protein signatures in CRC and NSCLC samples.
[0199] (A and B) Representative flow cytometry plots for tumor normal tissue infiltrating Treg cells and peripheral blood Treg cells analyzed for the expression of the indicated proteins.
[0200] FIG. **5**. Prognostic value of signature transcripts of tumor infiltrating Treg cells.
[0201] (A) Kaplan-Meier survival curve comparing the high and low expression of the tumor Treg signature transcripts (CCR8, MAGEH1, LAYN) normalized to the CD3G for the CRC (n=177) and NSCLC(n=263) studies. Univariate analysis confirmed a significant difference in overall survival curve comparing patients with high and low expression. Statistical significance was determined by the log-rank test. (CRC: p=0.05 for CCR8, p=1.48×10−3 for MAGEH1, p=2.1×10−4 for LAYN; NSCLC: p=0.0125 for CCR8, p=0.035 for MAGEH1, p=0.0131 for LAYN) Each table depicts the Kaplan Meier estimates at the specified time points. (B) Expression distributions of CCR8, MAGEH1 and LAYN according to tumor staging at the time of surgery in the cohort of CRC patients. See also FIG. **9**.
[0202] FIG. **6** related to FIG. **1**. Transcriptome analysis of tumor infiltrating lymphocytes.
[0203] (A) Representation of the sorting strategy of Treg cells infiltrating colorectal tumor or normal tissue.
[0204] (B) RNA-seq expression values (normalized counts) of FOXP3, TBX21 and RORC in CD4+ Th1, Th17 and Treg cells from CRC (C), NSCLC (L) or peripheral blood (PB) of healthy donors.
[0205] (C) RNA-seq normalized counts data for selected immune checkpoints and their ligands are shown as histogram plot. Cell population names are reported in the lower part of each graph, while gene names are shown in the upper part.
[0206] FIG. **7** related to FIG. **3**. Single-cell analysis of tumor infiltrating Treg cells.
[0207] Assessment of CD4+ Treg, Th1, Th17, Th2, CD8+ T cells and B cell markers expression (percentage of expressing cells) in single Treg cells purified from NSCLC and CRC.
[0208] FIG. **8** related to FIG. **4**. Comparison of BATF expression in CD4+ Treg vs Th17 cells.
[0209] BATF expression levels (RNA-seq normalized counts data) in CD4+ Treg and Th17 subsets isolated from tumor tissue or peripheral blood
[0210] FIG. **9** related to FIG. **5**. Expression levels of tumour-infiltrating Treg signature genes.
[0211] RNA-seq normalized counts data of three tumour-infiltrating Treg signature genes (MAGEH1 (panel A), LAYN (panel B) and CCR8 (panel C)) across listed cell populations.
[0212] FIG. **10**. Results of RT-PCR analysis done on cDNA from Tumor infiltrating Treg cells (L=NSCLC, C=CRC, −=ntc) with specific primers able to discriminate the different transcript isoforms annotated for SIRPG.

DETAILED DESCRIPTION OF THE INVENTION

Experimental Procedures

Human Primary Tissues

[0213] Primary human lung or colorectal tumors and non-neoplastic counterparts were obtained respectively from fifteen and fourteen patients who underwent surgery for therapeutic purposes at Fondazione IRCCS Ca' Granda, Policlinico or San Gerardo Hospitals (Italy).
[0214] Records were available for all cases and included patients' age at diagnosis, gender, smoking habit (for lung cancer patients), clinicopathological staging (Sobin et al., 2009), tumor histotype and grade (Table II). No patient received palliative surgery or neoadjuvant chemo- and/or radiotherapy. Informed consent was obtained from all patients, and the study was approved by the Institutional Review Board of the Fondazione IRCCS Ca' Granda (approval n. 30/2014).

[0215] Non-small-cell lung cancer (NSCLC) were cut into pieces and single-cell suspensions were prepared by using the Tumor Dissociation Kit, human and the gentleMACS™ Dissociator (Miltenyi Biotech cat. 130-095-929) according to the accompanying standard protocol. Cell suspensions were than isolated by ficoll-hypaque density-gradient centrifugation (Amersham Bioscience). Colorectal cancer (CRC) specimens were cut into pieces and incubated in DTT 0.1 mM (Sigma-Aldrich) for 10 min, then extensively washed in HBSS (Thermo Scientific) and incubated in 1 mM EDTA (Sigma-Aldrich) for 50 min at 37° C. in the presence of 5% CO2. They were then washed and incubated in type D collagenase solution 0.5 mg/mL (Roche Diagnostic) for 4 h at 37° C. Supernatants containing tumor infiltrating lymphocytes were filtered through 100 μm cell strainer, centrifuged and fractionated 1800×g for 30 min at 4° C. on a four-step gradient consisting of 100%, 60%, and 40% and 30% Percoll solutions (Pharmacia). The T cell fraction was recovered from the inter-face between the 60% and 40% Percoll layers.

[0216] CD4 T cell subsets were purified by FACS sorting using the following fluorochrome conjugated antibodies: anti-CD4 APC/Cy7 (Biolegend clone OKT4), anti-CD27 Pacific Blue (Biolegend, clone M-T271), anti-IL7R PE (Milteniy, clone MB15-18C9), anti-CD25 PE/Cy7 (eBioscience, clone BC96), anti-CXCR3 PE/Cy5 (BD, clone 1C6/CXCR3), anti-CCR6 APC (Biolegend, clone G034E3) and anti-CCR5 FITC (Biolegend, clone j418F1) using a FACSAria II (BD).

Flow Cytometry

[0217] To validate surface marker expression cells were directly stained with the following fluorochrome-conjugated antibodies and analyzed by flow cytometry: anti-CD4 (Biolegend, clone OKT4); anti-PD-L2 (Biolegend, Clone CL24F.10C12); anti-CD127 (eBioscience, clone RDR5); anti-BATF (eBioscience, clone MBM7C7), anti-GITR (eBioscience, clone eBIOAITR), anti-CD25 (Miltenyi, clone 4E3) and anti 4-1BB (eBioscience clone 4B4) anti CCR8(Biolegend clone L263G8) anti CD30 (eBioscience, clone Ber-H2) anti PD-L1 (Biolegend clone 29E.2A3) anti TIGIT (eBioscience, clone MBSA43) anti IL1 R2 (R and D clone 34141) IL21R (Biolegend clone 2G1-K12) anti OX40 (Biolegend clone Ber-ACT35). Intracellular staining was performed using eBioscience Foxp3 staining kit according to the manufactured's protocol (eBioscience cat 00-5523-00). Briefly cells were harvested and fixed for 30 min in fixation/permeabilization buffer at 4° C., and than stained with anti-FOXP3 antibody (eBioscience, clone 236A/E7) and anti-BATF (eBioscience clone MBM7C7) in permeabilisation buffer for 30 min at 4° C. Cells were then washed two times, resuspended in FACS washing buffer and analyzed by flow cytometry.

Suppression Assay.

[0218] 4×10.sup.4 carboxyfluorescein diacetate succinimidyl ester (CFSE)-labeled (1 μM) responders Naive.sup.+ T cells from healthy donors were cocultured with different E/T ratio with unlabeled CD127.sup.−CD25.sup.lowCD4.sup.+ T cells sorted from TILs or PBMCs of patients with CRC or NSCLC, using FACS Aria II (BD Biosciences), in the presence of CD11c.sup.+CD1c.sup.+dentritic cells as antigen-presenting cells and 0.5 mg/ml anti-CD3 (OKT3) mAb. Proliferation of CFSE-labeled cells was assessed by flow cytometry after 96 hr culture.

RNA Isolation and RNA Sequencing

[0219] RNA from tumor-infiltrating lymphocytes was isolated using mirVana Isolation Kit.

[0220] Residual contaminating genomic DNA was removed from the total RNA fraction using Turbo DNA-free (Thermo Fisher). The RNA yields were quantified using the QuantiFluor RNA System (Promega) and the RNA quality was assessed by the Agilent 2100 Bioanalyzer (Agilent). Libraries for Illumina sequencing were constructed from 50 ng of total RNA with the Illumina TruSeq RNA Sample Preparation Kit v2 (Set A). The generated libraries were loaded on to the cBot (Illumina) for clustering on a HiSeq Flow Cell v3. The flow cell was then sequenced using a HiSeq 2500 in High Output mode (Illumina). A paired-end (2×125) run was performed.

RNA-Seq Data Analysis

[0221] Raw .fastq files were analyzed using FastQC v0.11.3, and adapter removal was performed

using cutadapt 1.8. Cutadapt is run both for reverse and forward sequences with default parameters [-anywhere <adapter1>-anywhere <adapter2>-overlap 10-times 2-mask-adapter]. Adapter sequences used for libraries preparation are
TABLE-US-00004 Adapter1: (SEQ ID NO: 710) AGATCGGAAGAGCACACGTCTGAACTCCAGTCACNNNNNNATCTCGTATG CCGTCTTCTGCTTG Adapter2: (SEQ ID NO: 711) AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCC GTATCATT
[0222] Trimming was performed on raw reads using Trimmomatic (Bolger et al., 2014): standard parameters for phred33 encoding were used: ILLUMINACLIP (LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15), MINLEN parameter was set to 50.
[0223] Mapping and quantification: reads mapping to the reference genome (GRCh38) was performed on quality-checked and trimmed reads using STAR 2.4.1c: [STAR-genomeDir <index_star>-runThreadN <cpu_number>-readFilesin <trimmed>_R1.fastq.gz<trimmed>_R2_P.fastq.gz-readFilesCommand zcat]. The reference annotation is Ensembl v80. The overlap of reads with annotation features found in the reference .gtf was calculated using HT-seq v0.6.1. The output computed for each sample (raw read counts) was then used as input for DESeq2 analysis. Raw counts were normalized using DESeq2's function ‘rlog’, and normalized counts were used to perform and visualize Principal Component Analysis (PCA) results (using DESeq2's ‘plotPCA’ function).
[0224] Differential expression analysis: differential expression analyses of tumor-infiltrating CD4+ Treg/Th1/Th17 subsets vs. CD4+ Treg/Th1/Th17 from PBMC were performed using DESeq2. Upregulated/downregulated genes were selected for subsequent analyses if their expression values were found to exceed the threshold of 0.05 FDR (Benjamini-Hochberg correction).
Capturing of Single Cells, Preparation of cDNA and Single-Cell PCR
[0225] Treg cells from 5 CRC and 5 NSCLC specimens were isolated as previously described (See also Table II).Single cells were captured on a microfluidic chip on the C1 System (Fluidigm) and whole-transcriptome amplified. cDNA was prepared on chip using the SMARTer Ultra Low RNA kit (Clontech). Cells were loaded onto the chip at a concentration of 3-5E5 cells/ml, stained for viability (LIVE/DEAD cell viability assay; Thermo Fisher) and imaged by phase-contrast and fluorescence microscopy to assess the number and viability of cells per capture site. Only single, live cells were included in the analysis. For qPCR experiments, harvested cDNA was pre-amplified using a 0.2× pool of primers prepared from the same gene expression assays to be used for qPCR.
[0226] Pre-amplification allows for multiplex sequence-specific amplification 78 targets. In detail, a 1.25 μl aliquot of single cell cDNA was pre-amplified in a final volume of 5 μl using 1 μl of PreAmp Master Mix (Fluidigm) and 1.25 μl pooled TaqMan assay mix (0.2×). cDNA went through amplification by denaturing at 95° C. for 15 s, and annealing and amplification at 60° C. for 4 min for 20 cycles. After cycling, pre-amplified cDNA was diluted 1:5 by adding μl TE Buffer to the final 5 μl reaction volume for a total volume of 25 μl. Single-cell gene expression experiments were performed using the 96×96 quantitative PCR (qPCR) DynamicArray microfluidic chips (Fluidigm). A 2.25 μl aliquot of amplified cDNA was mixed with 2.5 μl of TaqMan Fast Adavanced Master Mix (Thermo Fisher) and 0.25 μl of Fluidigm's “sample loading agent,” then inserted into one of the chip “sample” inlets. A 2.5 μl aliquot of each 20×TaqMan assay was mixed with 2.5 μl of Fluidigm's “assay loading agent” and individually inserted into one of the chip “assay” inlets. Samples and probes were loaded into 96×96 chips using an IFC Controller HX (Fluidigm), then transferred to a BioMark real-time PCR reader (Fluidigm) following manufacturer's instructions. A list of the 78 TaqMan assays used in this study is provided below.
TABLE-US-00005 TABLE V Related to FIG. 3. List of TaqMan Probes and assay number used in RT-qPCR single-cell experiments Taqman Assays Numbers Assay Gene Name Number Gene Name Assay Number BCL2L1 Hs00235329_m1 ACP5 Hs00356261_m1 EOS Hs00223842_m1 BATF Hs00232390_m1 AHCYL1 Hs00198382_m1 SLC35F2 Hs00233850_m1 NFE2L3

Hs00852569_m1 LAX1 Hs00214948_m1 IL12RB2 Hs00155486_m1 CCR8 Hs00174764_m1 CD177 Hs00360669_m1 ADPRH Hs00153890_m1 OX40 HS00937194_g1 IKZF2 Hs00212361_m1 METTL7A Hs00204042_m1 C5F2RB Hs00166144_m1 ENTPD1 HS00969339_m1 NDFIP2 Hs00324851_m1 NFAT5 Hs00232437_m1 CADM1 Hs00942508_m1 CT9C Hs00175188_m1 ICOS Hs00359999_m1 SSH1 Hs00368014_m1 COL9A2 Hs00156712_m1 TMEM184C Hs00217311_m1 LTA Hs00236874_m1 HTATIP2 Hs03091727_m1 MAGEH1 Hs00371974_s1 HSDL2 Hs00953689_m1 IL21R Hs00222310_m1 FOXP3 Hs01085834_m1 S6TR3 Hs01066399_m1 IL2RA Hs00907778_m1 RNF145 Hs01066399_m1 LIMA1 Hs01033646_m1 LAPTM4B Hs00363282_m1 NAB1 Hs00428619_m1 GRSF1 Hs00909877_m1 ACSL4 Hs00244871_m1 ANKRD10 Hs00214321_m1 ERI1 Hs00405251_m1 NPTN Hs01033353_m1 FKEP1A Hs00356621_g1 HS3ST3B1 Hs00797512_s1 LEPROT Hs00956627_s1 TRAF3 Hs00936781_m1 NETO2 Hs00983152_m1 RRAGB Hs01099787_m1 VDR Hs00172113_m1 ZBT3S Hs00257315_s1 CSF1 Hs00174164_m1 TIGIT Hs00545087_m1 GITR Hs00188346_m1 TFRC Hs00951083_m1 IL1R2 Hs01030384_m1 JAK1 Hs01026982_m1 IL1R1 Hs00991010_m1 KSR1 Hs00300134_m1 LAYN Hs00379511_m1 ZNF202 Hs00411965_m1 THADA Hs00736554_m1 PTPRJ Hs01119326_m1 CTLA4 Hs00175480_m1 CHRNA6 Hs02563909_s1 CHST2 Hs01921028_s1 IL2RB Hs01081597_m1 CHST7 Hs00219871_m1 TBX21 Hs00203436_m1 LRBA Hs01032231_m1 RORC Hs01076112_m1 ETV7 Hs00903229_m1 CXCR5 Hs00540548_s1 LY75 Hs00982383_m1 CD8A Hs00233520_m1 ADAT2 Hs00699339_m1 CD8B Hs00174762_m1 GCNT1 Hs00155243_m1 PTGDR2 Hs00173717_m1 CASP1 Hs00354836_m1 CD19 Hs01047410_g1

[0227] Single-cell data analysis: The Quality Threshold in the BioMark™ Analysis software is a qualitative tool designed to measure the “quality” of each amplification curve. Basically, each amplification curve is compared to an ideal exponential curve and as the quality score approaches 1 the closer it is to ideal. The further the curve is from ideal, its quality score approaches 0. The default cutoff of 0.65 is an arbitrary value set by Fluidigm. Any curve above 0.65 passes. Any curve below, fails. Baseline correction was set on Linear (Derivative)[default]. Ct Threshold Method was set on Auto (Detectors). This method independently calculates a threshold for each detector on a chip. For clustering and downstream analysis, raw Cts have been converted to Log 2Exp by using a Limit of Detection (LOD) of 35, which corresponds to the last PCR cycle. Co-expression analysis has been performed by considering both CRC and NSCLC samples on those genes for which both FOXP3 and IL2RA were co-expressed at least to 2%. Gene's levels above the background were depicted as violin plots after log 2 scale transformation by ggplot2 (v. 2.1.10). The violin color gradient is the percentage of cells that are expressing the gene of interest and the upper bound of the color scale is the maximum percentage of cells that express a gene of the whole geneset.

[0228] Procedure for the removal of transcripts whose expression values are affected by the ‘dropout’ effect. Single-cell qPCR data are inherently noisy, and due the limitations of current technologies the expression patterns of a certain number of genes may be affected by the ‘dropout effect’. Inventors performed a gene selection procedure in order to take into account this ‘dropout’ effect and discard those genes whose expression values cannot be reliably used in a binary comparison (tumor-peripheral vs blood). Inventors fitted a number of parametric distributions to the ratios of detected genes on the total number of tumor cells (both NSCLC and CRC) and selected the reciprocal inverse Gaussian continuous random variable as best fit.

[0229] Inventors then calculated the median value of the fitted distribution and discarded those genes whose detection ratio is less than this threshold value (at least 8.4% of detection). Inventors reasoned that these genes are more likely to be affected by the ‘dropout’ effect. With this threshold inventors selected 45 genes for which a non-parametric T-test (Wilcoxon Mann Whitney test $p<0.05$) has been performed (by comparing tumor vs. peripheral blood samples).

Meta Analysis Kaplan-Meier and Stage Correlation

[0230] Statistical analysis was performed by using the R survival package (Therneau T. 2013).

Survival times were calculated as the number of days from initial pathological diagnosis to death, or the number of days from initial pathological diagnosis to the last time the patient was reported to be alive. The Kaplan-Meier (KM) was used to compare the high and low expression levels of the tumor-Treg cell signature transcripts in either CRC (GSE17536) and NSCLC (GSE41271) patients. For both studies annotation was normalized to four tumor stages (1,2,3,4). For study GSE41271 five patients were excluded due to incomplete or inaccurate annotation (GSM1012883, GSM1012884, GSM1012885, GSM1013100, GSM1012888), retaining a total of two hundred and sixty three patients. Patients from both studies were labeled as 'High' 'Low' whether or not their relative expression values exceeded a decision boundary (mean of the samples). Inventors define {umlaut over (x)}.sub.ij to denote the relative expression of the gene i for the n samples of the study normalized to the CD3 level:

[00001] $\tilde{x}_{i,j} = \frac{x_{i,j}}{x_{\mathrm{CD3}G,j}}; \quad i = (\mathrm{CCR8}, \mathrm{MAGEH1}, \mathrm{LAYN}) \quad j = 1, 2, \text{ .Math. }, n\text{samples}$

[0231] To classify a patient, a threshold on the {umlaut over (x)}.sub.ij is required and defined as

[00002] $T_{(\mathrm{UpperLower})} = \mathrm{median}(\tilde{x}_{i,j}) \pm \frac{\sigma(\tilde{x}_{i,j})}{10}$

where T.sub.(Upper,Lower) represent the upper and lower extreme of the decision boundary:

[00003] $$\begin{cases} \tilde{x}_{i,j} > T_{\mathrm{Upper}} & \mathrm{High} \\ \tilde{x}_{i,j} < T_{\mathrm{Lower}} & \mathrm{Low} \\ T_{\mathrm{Upper}} \leq \tilde{x}_{i,j} \leq T_{\mathrm{Lower}} & \mathrm{excluded} \end{cases}$$

[0232] Inventors examined the prognostic significance of tumor Treg cells transcripts by using log-rank statistics; a p-value of less than 0.05 was considered statistically significant. Since the log-rank test resulted in a p-value of less than 0.05, a post stage comparison by means of box plot representation was performed in order to evaluate the correlation degree between the expression level of the transcripts and tumor stages in the cohort of CRC patients. The annotation was normalized to four tumor stages (1,2,3,4).

ACCESSION NUMBERS

[0233] The accession numbers for the present data are as follows: ENA: PRJEB11844 for RNA-seq tumor and tissue infiltrating lymphocytes; ArrayExpress: E-MTAB-2319 for RNA-seq human lymphocytes datasets; ArrayExpress: E-MTAB-513 for Illumina Human BodyMap 2.0 project; GEO: GSE50760 for RNA-seq datasets CRC; GEO: GSE40419 for RNA-seq datasets NSCLC; GEO: GSE17536 for CRC expression profiling by array; and GEO: GSE41271 for NSCLC expression profiling by array.

Prediction of Surface-Exposed and Membrane-Associated Proteins

[0234] The probability of surface exposure of the proteins encoded by the genes of interest was determined by a combination of four different cell localization prediction algorithms: Yloc (Briesemeister et al, 2010), TMHMM (http://www.cbs.dtu.dk/services/TMHMM/), SignalP (http://www.cbs.dtu.dk/services/SignalP/) and Phobius (KAII et. al., 2007). In particular Yloc is a interpretable system offering multiple predictive models in animal version; inventors used both YLoc-LowRes predicting into 4 location (nucleus, cytoplasm, mitochodrion, secretory pathway) and Yloc-HighRes predicting into 9 locations (extracellular space, plasma membrane, nucleus, cytoplasm, mitochodrion, endoplasmic reticulum, peroxisome, Golgi apparatus, and lysosome).

[0235] TMHMM and SignalP were developed by the bioinformatic unit of the technical University of Denmark for the prediction of transmembrane helices and the presence and location of signal peptide cleavage sites in amino acid sequences, respectively. Phobius is a combined transmembrane topology and signal peptide predictor.

RT-PCR Analysis of Transcript Isoforms Expressed by Tumor-Infiltrating Regulatory T Cells (Treg Cells)

[0236] Total RNA was extracted from tumor Treg cells (NSCLC or CRC) using miRCURY RNA isolation kit (Exiqon) and 1 μg was reverse transcribed with iScript reverse transcription supermix

(BIORAD). Afterwards, 25 ng of cDNA were amplified with DreamTaq Green PCR Master Mix (ThermoScientific) using multiple gene-specific primers able to discriminate the different isoforms. PCR products were run on agarose gel. The expression of specific transcripts was assessed based on the expected band size.

Results

Tumor Infiltrating Tregs Cells Upregulate Immune Checkpoints and are Highly Suppressive

[0237] To assess the gene expression landscape of tumor infiltrating CD4.sup.+ T cells, the inventors isolated different CD4.sup.+ lymphocytes subsets from two different tumors, NSCLC and CRC, from the adjacent normal tissues, and from peripheral blood samples. From all these tissues, the inventors purified by flow cytometry (FIGS. **1**A and **6**A and **6**B) CD4.sup.+ Treg (36 samples from 18 individuals), Th1 (30 samples from 21 individuals) and Th17 (22 samples from 14 individuals) cells (Table I and Table II).

TABLE-US-00006 TABLE 1 Purification and RNA-Sequencing of Human Primary Lymphocyte Subsets Mapped Sorting Number of Reads Tissue Subset Phenotype Samples (M) NSCLC CD4+ Treg CD4.sup.+ CD127.sup.− CD25.sup.+ 8 587 CD4.sup.+ Th1 CD4.sup.+ CXCR3.sup.+ CCR6.sup.− 8 409 CD4.sup.+ Th17 CD4.sup.+ CCR6.sup.+ CXCR3.sup.− 6 206 CRC CD4.sup.+ Treg CD4.sup.+ CD127.sup.− CD25.sup.+ 7 488 CD4.sup.+ Th1 CD4.sup.+ CXCR3.sup.+ CCR6.sup.− 5 266 CD4.sup.+ Th17 CD4.sup.+ CCR6.sup.+ CXCR3− 5 308 Lung CD4.sup.+ Treg CD4.sup.+ CD127.sup.− CD25.sup.+ 1 (pool 73 (normal tissue) of 6) CD4.sup.+ Th1 CD4.sup.+ CXCR3.sup.+ CCR6.sup.− 1 (pool 76 of 6) Colon CD4.sup.+ Treg CD4.sup.+ CD127.sup.− CD25.sup.+ 7 404 (normal tissue) CD4.sup.+ Th1 CD4.sup.+ CXCR3.sup.+ CCR6.sup.− 6 352 CD4.sup.+ Th17 CD4.sup.+ CCR6.sup.+ CXCR3.sup.− 6 284 PB (healthy donor) CD4.sup.+ Treg CD4.sup.+ CD127.sup.− CD25.sup.+ 8 259 CD4.sup.+ Th1 CD4.sup.+ CXCR3.sup.+ CCR6.sup.− 5 70 CD4.sup.+ Th17 CD4.sup.+ CCR6.sup.+ CXCR3.sup.− 5 77 For each cell subsets profiled by RNA-sequencing tissue of origin, surface marker combinations used for sorting, number of profiled samples, as well as number of mapped sequencing reads are indicated. M, million; CRC, colorectal cancer; NSCLC, non-small cell lung cancer; PB, peripheral blood.

TABLE-US-00007 TABLE II Table II related to Table I. Patients information and histological analysis. For each cell subset profiled by RNA-sequencing, patient records are shown including: age at diagnosis, gender, smoking habit (for lung cancer patients), clinicopathological staging (TNM classification) tumor histotype and grade. For Treg cell isolated for qPCR experiment the same information are available, including also the number of live cells captured from each tumor and available for single-cell analysis. NSCLC PATIENTS LIST (RNA SMOKE SEQUENCING) (T)Th1 (T)Th17 (T)Treg (H)Th1 (H)Th17 (H)Treg HABIT STATUS GENDER PATIENT1 SQ_0342 PREVIOUS ALIVE M SMOKER >15 y PATIENT2 SQ_0339 PREVIOUS ALIVE M SMOKER <15 y PATIENT3 SQ_0365 SQ_0375 PREVIOUS ALIVE M SMOKER <15 y PATIENT4 SQ_0366 SQ_0374 SQ_0341 PREVIOUS ALIVE M SMOKER >15 y PATIENT5 SQ_0364 SQ_0373 SQ_0350 SQ_0351 SMOKER DEAD M PATIENT6 SQ_0358 SQ_0336 SQ_0350 SQ_0351 PREVIOUS ALIVE M SMOKER <15 y PATIENT7 SQ_0363 SQ_0376 SQ_0334 SQ_0350 SQ_0351 PREVIOUS ALIVE M SMOKER <15 y PATIENT8 SQ_0357 SQ_0337 SQ_0350 SQ_0351 PREVIOUS ALIVE M SMOKER WITH <15 y RELAPSE PATIENT9 SQ_0404 SQ_0408 SQ_0398 SQ_0350 SQ_0351 SMOKER ALIVE F PATIENT10 SQ_0403 SQ_0407 SQ_0396 SQ_0350 SQ_0351 PREVIOUS ALIVE F SMOKER >15 y NSCLC ADCA PATIENTS HISTO- SUBTYPE LIST (RNA TYPE (PRE- pTNM: pTNM: pTNM: SEQUENCING) AGE(y) MAJOR DOMINANT) GRADE T N M STAGE PATIENT1 84 SCC G3 2b 0 0 IIA PATIENT2 83 SCC G3 2a 0 0 IB PATIENT3 72 SCC G3 2 2 0 IIIA PATIENT4 79 SCC G3 2a 0 0 IB PATIENT5 66 SCC G3 3 2 0 IIIA PATIENT6 71 SCC G3 4 1 0 IIIA PATIENT7 78 SCC G3 2b 1 0 IB PATIENT8 77 ADCA SOLID G3 2 2 0 IIIA PATIENT9 69 ADCA SOLID G3 1a 0 0 IA PATIENT10 77 ADCA ACINAR G3 1a 0 0 IA NSLC = Non Small Cell Lung Cancer ADC = Adenocarcinoma SCC = Squamous Cell Carcinoma (T) = Tumor Sample (H) = Healthy Tissue

TUMOR INFILTRATING TREG FROM NSCLC HISTO- (SINGLE SMOKE TYPE ADCA SUBTYPE CELL qPCR) HABIT STATUS GENDER AGE(y) MAJOR PREDOMINANT PATIENT1 NEVER ALIVE F 65 ADCA ACINAR and SMOKER PAPILLARY PATIENT2 PREVIOUS ALIVE M 62 ADCA SOLID SMOKER <15 y PATIENT3 NEVER ALIVE F 63 ADCA ACINAR SMOKER PATIENT4 SMOKER ALIVE M 66 SCC PATIENT5 SMOKER ALIVE M 68 SCC TUMOR INFILTRATING TREG FROM NSCLC (SINGLE pTNM: pTNM: pTNM: CAPTURED CELL qPCR GRADE T N M STAGE SINGLE CELLS PATIENT1 G2 2a 0 0 IB 71 PATIENT2 G2 1b 0 0 IA 61 PATIENT3 G1 1a 0 0 IA 44 PATIENT4 G2 2a 0 0 IB 55 PATIENT5 G3 1b 0 0 IA 55 NSLC = Non Small Cell Lung Cancer ADC = Adenocarcinoma SCC = Squamous Cell Carcinoma (T) = Tumor Sample (H) = Healthy Tissue CRC PATIENTS LIST (RNA SEQUENCING) (T) Th1 (T) Th17 (T) Treg (H) Th1 (H) Th17 (H) Treg GENDER PATIENT1 SQ_0389 SQ_0386 SQ_0387 SQ_0388 M PATIENT2 SQ_0427 SQ_0434 SQ_0418 F PATIENT3 SQ_0423 SQ_0436 SQ_0411 M PATIENT4 SQ_0426 SQ_0437 SQ_0413 SQ_0428 SQ_0439 SQ_0417 M PATIENT5 SQ_0425 SQ_0412 SQ_0429 SQ_0441 SQ_0422 M PATIENT6 SQ_0424 SQ_0415 SQ_0431 SQ_0442 SQ_0421 M PATIENT7 SQ_0435 SQ_0416 SQ_0432 SQ_0438 SQ_0420 F PATIENT8 SQ_0414 F PATIENT9 SQ_0433 SQ_0430 SQ_0440 SQ_0419 M CRC PATIENTS HISTO- LIST (RNA TYPE pTNM: pTNM: pTNM: SEQUENCING) AGE(y) MAJOR GRADE T N M STAGE PATIENT1 76 ADC G2 3 1A 0 IIIB PATIENT2 68 ADC G2 3 0 0 IIA PATIENT3 80 ADC G2 4B 1B 0 IIIB PATIENT4 79 ADC G2 3 1A 0 IIIB PATIENT5 78 ADC G2 3 0 0 IIA PATIENT6 69 MUC — 3 1B 0 IIIB ADC PATIENT7 84 ADC G2 4B 0 0 IIC PATIENT8 75 MUC — 3 1C 0 IIIB ADC PATIENT9 54 ADC G2 2 0 0 I ADC = Adenocarcinoma MUC ADC = Mucinous Adenocarcinoma CRIB ADC = Cribrous Adenocarcinoma (T) = Subsets purified from Tumor Sample (H) = Subsets purified from Healthy Tissue TUMOR INFILTRATING TREG FROM HISTO- CRC (SINGLE TYPE pTNM: CELL qPCR) GENDER AGE(y) MAJOR GRADE T PATIENT1 M 64 ADC 2 3 PATIENT2 M 59 CRIB — 3 ADC PATIENT3 F 75 MUC — 4A ADC PATIENT4 M 71 ADC 1 3 PATIENT5 M 64 ADC 2 3 TUMOR INFILTRATING TREG FROM CRC pTNM: pTNM: CAPTURED (SINGLE CELL qPCR) N M STAGE SINGLE CELLS PATIENT1 0 0 IIA 62 PATIENT2 0 0 IIA 66 PATIENT3 2B 0 IIIC 65 PATIENT4 0 0 IIA 63 PATIENT5 0 0 IIA 64 ADC = Adenocarcinoma MUC ADC = Mucinous Adenocarcinoma CRIB ADC = Cribrous Adenocarcinoma (T) = Subsets purified from Tumor Sample (H) = Subsets purified from Healthy Tissue CRC: colorectal cancer; NSCLC: non-small cell lung cancer; (T): Tumor Sample; (H): Healthy Tissue; ADC: Adenocarcinoma; SCC: Squamous Cell Carcinoma; MUC ADC: Mucinous Adenocarcinoma.

[0238] To assess Treg cell function, inventors tested their suppressor activity and showed that Treg cells infiltrating either type of tumor tissues have a remarkably stronger suppressive activity in vitro compared to Treg cells isolated from the adjacent normal tissue and peripheral blood of the same patients (FIG. **1**B).

[0239] The polyadenylated RNA fraction extracted from the sorted CD4+ Treg, Th1, and Th17 cells was then analyzed by pair-end RNA sequencing obtaining about 4 billion mapped “reads” (Table 1). First, inventors interrogated RNA-sequencing data of CD4+ T cells infiltrating both CRC and NSCLC and their matched normal tissues, to quantitate mRNA expression of known immune checkpoints and their ligands. Second, inventors analyzed RNA-seq data of CRC and NSCLC, as well as of normal colon and lung samples.

[0240] Inventors found that several immune checkpoints and their ligands transcripts were strikingly upregulated in tumor infiltrating Treg cells compared to both normal tissue and peripheral blood-derived Treg cells, as well as to T and B lymphocyte subsets purified from peripheral blood mononuclear cells (PBMCs) (FIGS. **10**C and **60** and Table Ill).

TABLE-US-00008 Treg_Tumor_ Treg_Tumor_ Treg_Tissue_ Treg_Tissue_ Treg healthy GENE Infiltrating Infiltrating Inflitrating Inflitrating Peripheral NAME CRC NSCLC Colon Lung Blood ADORA2A 14.69 24.06 17.97 44.84 18.52 BTLA 554.04 742.11 389.51 208.76 108.2 BTNL2 0

0.14 0.29 0 0.75 (BTLN2) C10orf54 779.38 872.36 555.47 1405.63 1111.37 (VISTA) CD160 58.39 38.24 51.87 34.54 36.55 CD200 268.39 283.21 282.05 104.64 99.59 CD200R1 95.89 136.08 81.36 349.99 59.03 CD244 34.46 31.21 29.59 128.35 47.8 CD27 710.13 1068.55 583.58 496.38 468.93 CD274 1050.94 645.66 576.59 390.71 120.19 (PD-L1) CD276 16.85 72.3 10.44 65.98 3.61 CD28 4770.41 4585.17 5446.29 3687.01 5179.32 CD40 112.04 161.29 80.64 93.3 34.71 CD40LG 135.51 143.07 360.09 418.55 104.22 CD44 13049.36 8518.98 13513.69 19851 16013.71 CD48 346.61 489.78 494.58 594.83 1523.63 CD70 426.35 269.38 318.97 249.48 101.67 CD80 632.12 483.34 318.48 269.06 114.41 CD86 29.52 78.86 52.72 278.86 3.87 CTLA4 6798.82 10378.3 4810.74 5340.06 4806.23 HAVCR2 577.57 633.27 265.84 487.62 49.81 (TIM-3) HHLA2 3.41 3.66 4.47 9.28 12.7 ICOS 6830.94 7339.08 4119.2 5211.71 3398.28 ICOSLG 58.02 8.86 59.13 33.5 76.5 (B7RP1) IDO1 3.86 83.81 9.51 5.15 2.36 IDO2 0.22 2.25 1.41 5.15 1.58 KIR3DL1 0.38 0.43 0.28 4.64 0.9 (KIR) LAG3 705.14 1956.22 2181.52 1505.63 127.02 LAIR1 277.06 194.09 551.94 874.72 346.22 LGALS9 1175.81 1530.47 1160.89 1593.26 592.56 (Galectin-9) NRP1 7.38 36.24 8.89 106.7 8.59 PDCD1LG2 214.51 223.04 61.89 25.77 12.12 (PD-L2) PDCD1 467.22 496.56 405.01 676.27 111.26 (PD1) TIGIT 14821.45 14747.79 10986.74 4901.41 4611.14 TMIGD2 28.38 16.64 78.3 75.77 71.27 TNFRSF14 2230.85 2677.32 2297.43 2675.7 2274.82 (HVEM) TNFRSF18 4038.86 4078.14 2871.78 3071.57 333.36 (GITR) TNFRSF25 5236.86 4188.61 4986.56 5111.71 3587.58 TNFRSF4 4222.16 4642.56 2873.16 2992.18 400.56 (OX40) TNFRSF8 155.59 430.23 115.57 208.24 30.89 (CD30) TNFRSF9 2921.72 3128.82 898.69 1739.13 502.86 (4-1BB) TNFSF14 148.57 183.77 223.49 421.12 105.12 (LIGHT) TNFSF15 1.58 3.75 0.89 25.77 1.23 TNFSF18 0.4 1.11 0.53 0 0.45 TNFSF4 110.82 136.82 100.95 98.97 16.33 (OX40LG) TNFSF9 26.79 19.48 19.72 29.9 7.41 (CD137L) VTCN1 1.12 4.49 1.48 1.55 2.65 (B7-H4)

[0241] RNA-seq normalized counts data for selected immune checkpoints genes and their ligands in all the subsets analyzed.

[0242] These findings highlight the specific expression patterns of immune checkpoints and their ligands in tumor infiltrating Treg and effector cells and suggest that their functional relevance should be investigated directly at tumor sites.

Tumor-Infiltrating Treg Cells Express a Specific Gene Signature

[0243] The inventors then asked whether tumor infiltrating Treg cells could be defined by specific gene expression patterns.

[0244] To identify signature transcripts of tumor-infiltrating Treg cells, the inventors included in the expression pattern analyses the transcriptome dataset they previously obtained from different T and B lymphocyte subsets purified from PBMCs (Ranzani et al., 2015). In so doing, the inventors obtained a signature of 328 transcripts whose expression is higher in tumor infiltrating Treg cells (Wilcoxon Mann Whitney test $p<2.2\times10-16$) (FIG. **2**, and Table IV compared to the other lymphocyte subsets purified from non-tumoral tissues and from PBMCs of healthy or neoplastic patients.

TABLE-US-00009 Treg_Tumor_ Treg_Tumor_ Treg_Tissue_ Treg_Tissue_ Treg healthy Gene Infiltrating Infiltrating Inflitrating Inflitrating Peripheral Name CRC NSCLC Colon Lung Blood AC019206.1 15.41 8.72 12.89 12.04 29.46 ACAA2 305.76 499.02 497.41 526.58 614.28 ACOT9 918.3 803.71 1361.82 2180.66 1272.07 ACOX3 183.48 384.73 469.06 506.97 439.27 ACP5 267.7 837.72 859.77 1872.29 1483.27 ACSL4 1154.87 1384.88 1903.56 2170.94 2043.91 ACTA2 86.65 270.74 108.76 234.86 232.15 ACTG2 10.69 6.16 22.68 21.11 36.14 ADAM10 2378.26 3051.7 2545.29 3600.38 3167.56 ADAT2 927.45 1272.17 1214.4 2094.25 3103.21 ADPRH 136.34 460.61 352.57 836.7 718.74 AHCYL1 914.19 1271.5 1269.55 1835.94 1711.94 AHCYL2 305.15 570.67 525.24 790.1 856.25 AKAP5 174.24 264 358.75 709.28 535.97 AKIP1 261.47 273.85 225.25 436.84 360.48 ANKRD10 2251.92 3433.73 2805.08 4192.8 4672.81 ARHGEF12 1371.05 2064.05 1536.04 3069.77 2637.79 ARHGEF4 19.42 71.47 28.87 195.02 252.84 ARL6IP5 3008.69 4385.74 4051.43 4983.16 4712.48 ARNTL2 20.4 201.3 281.95 560.77 445.13 ATP13A3 3776.14 4020.7 4688.02 6688.94 6967.94 ATP2C1 1491.87 1399.81 1553.57 2029.41 1819.78 AURKA 24.56 50.12

79.89 66.37 87.07 BATF 820.97 3325.93 1698.92 5052.64 2727.65 BCL2L1 212.64 478.8 537.61 554.11 892.28 BIRC5 14.74 20.27 20.62 25.03 44.99 C17orf96 19 174.31 159.79 239.88 377.03 C5orf63 146.45 201.44 112.88 228.2 357.09 CABLES1 59.04 196.68 125.77 473.94 386.73 CACNB2 67.43 50.49 40.21 169.83 105.62 CADM1 113.76 602.72 115.46 1766.12 901.32 CALM3 2474.48 2829.3 2675.18 2954.03 4107.03 CARD16 370.31 696.36 493.29 1220.7 823.89 CARD17 41.87 96.94 54.12 101.19 132.95 CASP1 925.29 1453.84 1521.09 2028.95 1980.45 CASQ1 52.11 31.21 24.74 135.08 174.95 CCNB2 18.28 27.62 34.02 51.57 58.08 CCR8 255.66 578.27 1355.63 3127.33 2069.11 CD177 2.36 204.74 299.99 718.58 470.27 CD27 468.93 583.58 496.38 710.13 1068.55 CD274 120.19 576.59 390.71 1050.94 645.66 CD7 1622.12 6900.01 2829.82 9053.96 6919.59 CDCA2 19.24 35.09 49.48 68.21 49.95 CDH24 57.67 57.11 89.69 148.93 105.02 CDK6 602.97 2175.36 2463.85 3580.4 3238.58 CEACAM1 360.01 340.84 326.28 381.79 732.86 CENPM 43,72 39.12 61.85 72.94 61.32 CEP55 56.18 88.17 223.71 220.17 273.64 CGA 1.08 13.59 22.68 334.28 9.73 CHRNA6 14.46 218.49 67.52 336.38 504.28 CHST11 1822.7 2085.92 2806.11 2790.19 2535.23 CHST2 75.46 218.75 156.7 458.24 604.97 CHST7 141.3 341.87 426.79 1087.21 333.3 CIT 89.25 105.13 155.15 150.2 262.67 CLNK 153.06 288.36 248.96 340.12 528.54 CNIH1 1028.31 1005.46 935.03 2336.95 1101.87 COL9A2 149.87 278.77 357.72 889.47 805.72 CORO1B 481.34 667.37 861.83 774.65 1040.47 COX10 305.31 399.33 397.93 447.17 612.29 CRADD 77.04 155.66 277.31 394.31 306.61 CREB3L2 739.04 1289.66 1415.94 2984.54 2590.37 CSF1 313.09 1629.13 1609.75 2204.79 3288.67 CSF2RB 1069.75 1275.49 1290.69 2036.76 2531.99 CTLA4 4806.23 4810.74 5340.06 6798.82 10378.3 CTSC 1026.76 2196.93 2514.88 3030.74 2767.27 CTTNBP2NL 85 200.53 248.45 500.75 267.16 CX3CR1 9.57 63.99 123.71 341.79 293.28 CXCL13 1.07 255.23 1145.33 1270.98 11433.26 CYB5B 714.26 1129.39 947.4 1156.4 1221.22 CYP7B1 9.83 210.33 29.38 186.99 161.17 DCPS 153.25 210.26 210.82 191.31 271.71 DFNB31 561.87 1636.56 1727.79 4251.83 2526.15 DIRAS3 1.9 4.59 3.61 26.01 35.64 DLGAP5 7.89 14.46 20.62 27.41 49.7 DNPH1 160.15 650.05 321.13 683.55 576.77 DOC2B 10.47 3.42 5.15 14.23 238.86 DPYSL2 208.98 189.08 580.4 591.32 618.42 EBI3 7.47 103.59 56.7 148.96 200.74 ECEL1 3.7 150.7 34.02 199.17 794.51 EGLN1 977.29 969.32 1021.11 1381.2 1271.06 EML2 861.51 1601.25 1643.25 2156.04 1957.43 ENTPD1 752.88 2078.17 1447.38 4321.79 4162.57 ERI1 354.33 862.86 932.45 1200.06 1070.15 ETFA 414.08 586.15 534.01 615.35 689.14 ETV7 93.62 511.26 361.85 728.85 1111.55 EVA1B 21.39 35.63 26.8 42.86 47.36 F5 2343.39 2346.94 2499.41 4868.41 4729.97 FAAH2 244.19 431.76 209.27 737.44 699.42 FAIM2 15.05 33.47 57.21 69.26 117.28 FAM184A 192.41 742.47 525.24 706.33 891.02 FAM19A2 311.38 204.56 302.57 264.46 748.09 FAM98B 314.26 664.69 491.22 698.92 657.42 FAS 2337.14 5167.46 2712.81 5982.39 3656.21 FBXO45 460.56 783.06 631.43 964.13 894.23 FCRL3 1161.64 1997.02 938.63 3281.36 2699.01 FKBP1A 733.83 1240.62 1174.19 1377.67 1578.09 FLNB 1671.04 1363.04 1394.81 3395.38 2307.44 FLVCR2 69.84 579.55 388.13 744.8 528.01 FNDC3B 377.47 501.27 506.17 1111.07 531.12 FOXA1 2.7 11.87 17.01 70.68 18.22 FOXM1 56.39 74.94 108.24 88.16 125.31 FOXP3 6586.98 10713.12 6060.66 13483.77 11472.41 FUCA2 107.56 175.46 160.82 249.54 315.45 GADD45A 745.14 1431.9 884.51 3681.24 1396.98 GCNT1 99.22 632.16 608.75 1133.62 845.83 GK 637.31 1994.73 2430.34 5200.55 2065.35 GLB1 563.96 819.22 873.17 1077.84 854.94 GLCCI1 1557.57 3211.73 1753.04 3189.77 2909.06 GLDC 19.25 20.56 25.26 31.21 74.61 GLRX 1213.06 1251.64 1512.85 1764.61 1872 GNG4 5.08 79.18 64.43 197.1 343.93 GNG8 11.94 63.28 10.82 67.63 175.16 GRSF1 1277.4 1725.67 1397.9 2899.76 2343.4 GSK3B 1099.5 1267.18 1208.73 1333.16 1454.67 GTF3C6 313.17 579.04 445.86 617.48 597.55 GTSF1L 13.67 20.36 15.46 44.6 99.03 HADHB 1179.61 1207.14 1287.59 1396.89 1521.16 HAP1 92.39 180.51 74.22 292.97 577 HAVCR2 49.81 265.84 487.62 577.57 633.27 HECW2 17.63 98.93 38.66 111.21 177.5 HIBCH 124.32 290.04 226.8 348.34 332.88 HIVEP3 358.34 649.68 893.27 1091.96 1316.89 HJURP 8.55 18.52 15.98 27.13 39.99 HOXA1 16.66 15.22 14.95 25.57 44.75 HPRT1 442.58 532.66 542.25 811.75 724.15 HPSE 248.88 676.54 515.45 674.09 754.04 HS3ST3B1 1222.43 1930.88 1980.87 2609.49 2431.83 HSDL2 242.56 611.72 285.56 785.27 921.97 HTATIP2

567.61 1439.29 997.4 3285.86 1576.24 ICA1 94.65 371.57 113.91 487.68 411.64 ICOS 3398.28 4119.2 5211.71 6830.94 7339.08 IGFLR1 67.43 78.13 92.78 108.12 185.13 IKZF2 6061.48 6317.6 4919.45 9983.52 8551.49 IKZF4 1422.66 2362.49 1258.21 3745.25 3958.19 IL12RB2 120.8 369.84 509.78 835.92 877.51 IL17REL 9.74 23.21 34.02 52.62 57.04 IL1R1 506.51 9670.81 2766.42 7852.18 5585.89 IL1R2 41.72 1225.4 526.79 2117.34 1793.21 IL1RL1 17.37 135.26 44.33 715.42 71.67 IL1RL2 8.65 76.53 28.35 74.81 59.47 IL21R 708.61 1355.83 1715.93 3092.3 3514.36 IL2RA 5244.31 9685.38 5627.68 11454.42 12731.31 IL2RB 6716.4 14249.6 12502.75 17733 18564.35 IL32 4332.08 13202.73 9755.92 11766.98 13883.45 IL7 117.66 230.78 165.97 257.71 178.1 INPP1 124.25 497.01 312.88 458.2 487.93 INPP5F 787.92 2172.55 830.9 2189.48 1549.46 ISOC1 233.44 329.49 400.5 514.43 335.93 ITFG1 313.34 324.11 402.05 396.94 511.86 JAK1 10779.78 11919.66 10072.4 17755.9 11521.32 JAKMIP1 291.14 387.49 1063.89 756.36 953.47 KAT2B 3145.05 3910.01 4756.57 5520.88 4632.76 KIF14 20.18 25.43 31.96 36.73 59.61 KIF15 20.64 29.67 51.03 41.9 68.63 KIF20A 9.84 14.93 7.22 20.97 32.72 KLHDC7B 131.39 211.42 188.65 245.3 394.73 KSR1 837.87 1569.86 1176.77 2241.36 1847.72 LAPTM4B 86.42 369.78 181.44 938.88 738.38 LAX1 1135.24 1155.91 1406.15 1721.7 1854.78 LAYN 441.73 796.76 859.25 2650.24 1681.25 LEPR 58.77 130.22 129.38 137.47 237.88 LEPROT 614.73 860.55 676.79 1044.66 1296.13 LHFP 1.58 10.38 9.79 18.09 63.16 LIMA1 404.55 727.57 1017.5 1064.46 1570.15 LMCD1 115.76 104.74 112.37 257.92 404.7 LOC388813 7.42 45.99 28.87 86.3 60.63 LRG1 17.67 61.54 46.39 71.6 78.3 LRRC61 98.78 291.45 138.66 292.51 314.79 LTA 214.07 516.57 270.61 351.26 747.01 LXN 67.37 91.06 75.77 114.23 133.43 LY75 249.92 970.85 680.91 1302.79 1624.82 MAGEH1 461.13 1349.51 448.96 2800.36 3719.29 MALT1 3362.14 3568.46 2743.74 5892.86 4776.24 MAP1LC3A 70,92 110,44 119,07 272,07 169,3 MAP3K5 1865.12 2189.99 1787.06 2822.55 2265.54 MAST4 1053.08 2239.36 2198.39 3373.36 1855.42 MAT2B 2305.62 4050.5 2959.2 4435.41 4159.25 MCCC2 737.75 875.78 873.69 1018.1 1245.79 MELK 28.77 50.08 83.5 72.28 83.06 METTL7A 280.99 442.99 385.04 845.09 1671.74 METTL8 318.99 882.21 377.82 880.99 1413.12 MGME1 236.76 332.08 342.77 400.19 552.69 MGST2 54.22 87.18 69.59 147.04 148.13 MICAL2 354.6 1601.79 1813.35 1910.22 3188.92 MINPP1 85.19 204.32 211.85 243.22 290.02 MKI67 192.68 206.77 518.03 372.61 650.04 MREG 120.75 119.91 226.28 229.41 325.33 MYL6B 122.13 182.71 107.73 174.22 252.52 MYO5C 95.68 122.36 157.21 130.81 347.49 NAB1 508.21 973.74 1261.31 1831.77 1227.51 NCALD 111.73 163.32 272.67 283.43 370.26 NCAM1 7.88 58.27 39.69 207.45 213.23 NCF4 509.63 630.55 880.39 894.67 1176.84 NCOA1 2088.38 2062.57 1941.7 2367.54 2618.11 NDFIP2 77.99 529.73 618.54 829.53 987.25 NEMP2 382.56 478.4 475.76 565.18 634.41 NETO2 145.84 559.95 773.69 1490.82 1137.73 NEURL3 4.04 29.74 12.37 24.02 35.49 NFAT5 2075.17 3880.92 3923.6 4786.04 5295.06 NFE2L3 279.28 590.19 560.29 743.24 1114.26 NFYC 588.49 713.51 756.16 733.52 798.27 NHS 7.27 18.73 55.15 60.16 159.44 NPTN 525.86 838.02 897.91 1007.87 969.1 NTNG2 117.04 296.81 534.52 669.43 1001.58 NTRK1 20.85 27.9 155.15 88.29 161.78 NUSAP1 199.28 266.11 445.86 635.51 365.17 NXT2 221.6 263.39 226.8 285.15 302.01 OSBP2 111.03 89.82 127.83 195.47 244.93 PAK2 4621.62 6173.86 5024.6 7194.78 6376.28 PAM 582.52 904.05 1069.56 1365.03 1631.64 PANX2 3.7 76.02 15.46 97.12 71.72 PAQR4 16.99 46.54 62.37 92.6 65.27 PARD6G 55.86 172.18 249.99 546.52 182.4 PARK7 1271.06 1563.96 1283.47 1764.8 1764.91 PCTP 49.2 173.47 163.4 253.27 270.62 PDCD1LG2 12.12 61.89 25.77 214.51 223.04 PDGFA 6.19 38.74 159.79 154.17 153.03 PEX3 179.31 239.78 205.66 326.61 291.17 PGM2 316.91 419.51 454.63 471.89 487.85 PHKA1 8.59 19.98 28.87 107.79 109.7 PIGU 147.54 205.18 184.53 220.25 265.12 PLA2G4C 22.16 128.81 65.98 245.65 159.6 PPM1G 1974.96 2324.16 2563.85 2751.69 2598.5 PRDX3 466.56 854.12 745.34 890.58 1052.67 PRKCDBP 4.45 6.8 19.07 28.51 27.92 PROB1 53.7 140.39 109.79 177.19 272.89 PTGIR 96.17 147.61 107.21 214.61 449.25 PTP4A3 134.06 262.63 463.39 340.08 667.84 PTPRJ 2654.92 3999.84 5584.38 6101.63 7239.3 PTTG1 211.97 198.56 236.59 302.53 335.68 RAB15 160.6 470.25 302.05 420.06 519.4 RAD51AP1 29.89 46.33 40.21 49.23 51.73 RASAL1 18.87 53.37 50 87.38 238.78 RBKS 67.62 56.45 133.5 141.16 85.46 RCBTB1 1154.33 1312.01

1131.41 1960.76 1384.84 RDH10 194.04 311.58 467.51 658.5 1448.57 REXO2 487.9 832.35 648.44 852.58 987.43 RFK 378.31 396.91 292.26 460.78 452.8 RGS1 16547.6 15176.27 18057.75 23425.18 17168.17 RHOC 78.07 230.17 207.21 317.85 290.86 RMI2 19.46 76.58 39.69 70.44 73.47 RNF145 1625.11 3074.78 2117.47 4417.29 3266.94 RNF207 41.75 469.3 314.94 723.56 765.87 RRAGB 281.49 274.98 196.9 384.81 506.1 RYBP 1861.27 2273.72 2496.32 3178.31 2818.02 SEC14L6 6.42 86.23 27.32 179.47 274.97 SEC24A 718 917.25 1157.7 1259.04 1062.95 SECTM1 69.01 1347.35 725.75 2354.1 1511.04 SEPT3 15.6 59.23 49.48 149.11 244.4 SGPP2 428.14 656.73 364.94 1001.71 809.92 SH3RF2 20.9 18.3 65.98 98.4 196.34 SIRPG 433.99 605.49 317 575.41 1245.12 SLC16A1 947.47 1385.08 1532.43 2050.74 1460.73 SLC25A12 246.72 323.6 423.18 406.15 498.91 SLC35E3 385.3 451.16 370.09 582.86 653.13 SLC35F2 378.22 795.55 688.64 1130.81 880.5 SLC41A1 1194.29 1119.86 1164.92 1401.41 1630.88 SLC41A2 13.45 356.73 114.95 482.48 395.27 SMAD1 15.34 53.93 30.41 63.54 87.46 SMS 565.6 760.65 719.57 818.12 735.99 SNAP47 310.71 503.77 577.82 690.31 696.18 SOCS2 245.77 405.76 463.39 605.25 611.78 SOX4 128.76 244.57 218.04 1205.78 715.01 SPATA24 38.86 77.02 36.6 66.43 94.41 SPATC1 7.97 10.96 19.59 61.51 55.84 SPATS2L 366.98 891.61 1172.13 1430.11 1531.61 SSH1 1890.01 3432.55 2771.06 4390.36 4552.26 SSTR3 230.28 248.12 341.74 240.77 901.25 STAC 11.63 48.36 39.69 75.94 71.4 STARD7 2415.01 3185.95 3024.66 3809.46 3445.47 STRIP2 103.39 1002.96 540.19 716.49 1192.77 SYT11 1078.51 1733.37 2080.36 2110.18 2818.39 TADA3 677.14 893.74 852.04 880.43 1189.01 TBC1D8 53.89 374.1 265.97 817.36 1087.39 TDRD3 461.34 383.25 520.09 584.64 643.84 TFRC 3608.04 4612.18 5640.05 8107.35 10082.21 THADA 1102.51 1505.13 1467.48 3472.21 3171.99 TIGIT 4611.14 10986.74 4901.41 14821.45 14747.79 TM9SF2 2048.03 2689.14 2665.91 2935.98 3358.4 TMA16 172.88 180.92 137.11 304.24 192.53 TMEM140 273.98 640.28 574.73 917.16 691 TMEM184C 520.19 508.83 599.98 1170.37 519.43 TMOD1 14.75 72.22 32.47 150.93 89.62 TMPRSS3 70.84 352.78 321.64 540.8 1106.85 TMPRSS6 113.53 548.87 265.97 698.41 985.34 TNFRSF18 333.36 2871.78 3071.57 4038.86 4078.14 TNFRSF4 400.56 2873.16 2992.18 4222.16 4642.56 TNFRSF8 30.89 115.57 208.24 155.59 430.23 TNFRSF9 502.86 898.69 1739.13 2921.72 3128.82 TNIP3 28.73 485.83 213.91 324.53 419.8 TOR4A 141.27 291.3 346.9 358.98 326.51 TOX2 237.46 860.48 490.71 861.08 1264.13 TP73 7.86 31.27 39.69 78.27 93.99 TPMT 357.13 354.93 305.66 480.15 519.82 TPP1 2589.92 6024.92 4380.81 7164.96 6236.83 TPX2 106.25 89.08 184.02 150.35 202.77 TRAF3 1140.85 3231.25 2706.11 4078.84 3554.01 TRIB1 927.27 1820.64 1482.95 2402.58 1469.85 TRIM16 160.05 115.2 121.13 240.55 210.13 TSPAN17 709.59 1721.26 1322.64 1685.38 1865.69 TSPAN5 372.4 1167.46 723.69 1230.67 1398.7 TST 3.8 26.32 26.8 39.78 41.65 TTBK1 13.41 164.27 99.48 380.69 460.64 TTC22 237.9 386.91 323.19 483.96 451.61 TWIST1 4.21 94.46 21.65 95.32 195.78 UGP2 1950.41 3283.79 2562.82 3399.18 2864.71 USP51 48.1 133.95 28.87 233.48 291.46 UXS1 1661.1 2156.16 1600.47 2614.66 1914.74 VANGL1 97.19 192.58 248.96 263.46 289.05 VDR 123 992.41 1771.6 2616.68 3656.18 VWA5A 426.29 550.67 373.7 604.53 739.57 WDHD1 101.74 126.37 140.2 136.76 193.58 WDTC1 1220.3 3855.35 2029.33 4398.54 3774.61 WSB1 2837.49 3876.77 4697.29 5090.18 5383.33 XKRX 16.06 71.84 90.2 115.05 101.81 YIPF1 310.29 351.68 285.04 354.44 456.27 YIPF6 342.01 687.07 705.14 1078.09 793.2 ZBED2 87.53 94.86 522.15 230.51 1238.63 ZBTB38 1986.89 5405.41 3134.97 6174.05 4680.43 ZC3H12C 123.76 159.39 518.54 1191.95 985.54 ZG16B 3.42 17.03 15.46 32.31 32.59 ZMAT3 529.91 925.46 822.66 1077.17 1234.3 ZMYND8 585.94 675.31 711.84 850.29 1131.01 ZNF280C 181.86 444.81 326.28 635.21 467.78 ZNF280D 698.54 973.93 616.48 1061.55 1290.04 ZNF282 374.36 1273.4 2253.55 2562.43 3165.99 ZNF334 6.95 26.52 17.53 40.03 100.33 ZWINT 60.55 73.28 101.03 87.1 105.4

[0245] Altogether, the data show that Treg cells display the most pronounced differences in transcripts expression among CD4+ T cell subsets infiltrating normal and tumor tissues.

[0246] The inventors defined a subset of signature genes that describe the specific gene expression profile of tumor infiltrating Treg cells.

Gene Signature of Tumor-Infiltrating Treg Cells is Present in Primary and Metastatic Human

Tumors

[0247] The inventors then looked at the single cell level for the differential expression profile of signature genes of tumor infiltrating Treg cells. The inventors isolated CD4+ T cells from CRC and 5 NSCLC tumor samples as well as from 5 PBMCs of healthy individuals (Table II), purified Treg cells, and using an automated microfluidic system (Cl Fluidigm) captured single cells (a total of 858 Treg cells: 320 from CRC and 286 from NSCLC; 252 from PBMCs of healthy individuals). The inventors then assessed by high throughput RT-qPCR (Biomark HD, Fluidigm) the expression of 79 genes selected among the highly expressed (>10 FKPM) tumor Treg cell signature genes (FIGS. **3**A, **3**C and **7**). Notably, it was found that the vast majority (75 over 79; 95%) of the tumor-infiltrating Treg cell signatures were co-expressed with bona fide Treg cell markers (i.e., FOXP3+ and IL2RA) (FIG. **3**B). The percentage of co-expression between these Treg cell markers and the 79 genes selected among the tumor-infiltrating-Treg-cell signature genes ranged between 81% of TIGIT and 0.59% of CGA (FIG. **3**B). The expression of Treg signature genes in the RNA-seq of the whole Treg cell population correlated with the percentage of single cells expressing the different genes (FIG. **3**C). In order to reduce the “drop-out” effect of the single cell data (i.e., events in which a transcript is detected in one cell but not in another one because the transcript is ‘missed’ during the reverse-transcription step) (Kharchenko et al., 2014), a threshold (median value t=8.4%) was defined based on the expression distribution for each transcript and discarded genes below this threshold. The forty-five signature transcripts of tumor infiltrating Treg cells detected above this threshold were in most cases significantly over-expressed in Treg cells from both tumors (39 over 45, 87%; Wilcoxon Mann Whitney test $p<0.05$) or in one tumor type (43 over 45, 96%; FIG. **3**D). Homogeneity of the purified tissue infiltrating Treg cells can be affected by the carry-over of cells from other lymphocyte subsets. To quantitate this possible contamination, the single cell RT-qPCR analyses of Treg cells was performed including markers specific for other lymphocytes subsets (i.e., Th1, Th2, Th17, Tfh, CD8 T cells, B cells) (FIG. **7**). Our data showed that only a very low fraction of the purified single cells displayed markers of lymphocytes subsets different from Treg cells (FIG. **7**).

[0248] The overlap between the signature genes in the CRC and NSCLC infiltrating Treg cells (FIG. **2**) prompted us to assess whether this signature were also enriched in Treg cells infiltrating other tumors. RNA was thus extracted from Treg cells infiltrating breast cancer, gastric cancer, brain metastasis of NSCLC, and liver metastasis of CRC. It was found by RT-qPCR that tumor infiltrating Treg signatures genes were mostly upregulated also in these tumors (FIG. **3**E).

[0249] Overall these data show that the tumor-infiltrating Treg cell signature genes are co-expressed at single cell level with FOXP3 and IL2RA and that several primary and metastatic human tumors express the tumor-infiltrating Treg cell signature.

Gene Signature of Tumor Infiltrating Treg Cells is Translated in a Protein Signature

[0250] The inventors then assessed at the single cell level by flow cytometry the protein expression of ten representative signature genes present in CRC and NSCLC infiltrating Treg cells, adjacent normal tissues, and patients PBMCs. Of the ten proteins, two are proteins (OX40 and TIGIT) whose relevance for Treg cells biology has been demonstrated (Joller et al., 2014; Voo et al., 2013), seven are proteins (BATF, CCR8, CD30, IL-1 R2, IL-21R, PDL-1 and PDL-2) whose expression has never been described in tumor-infiltrating Treg cells, and one protein, 4-1BB, is a co-stimulatory receptor expressed on several hematopoietic cells, whose expression on Treg cells has been shown to mark antigen-activated cells (Schoenbrunn et al., 2012). Our findings showed that all these proteins were upregulated (FIGS. **4**A and **4**B), at different extent, in tumor infiltrating Treg cells compared to the Treg cells resident in normal tissues.

[0251] Altogether, our data show there is a molecular signature of tumor infiltrating Treg cells, which can be detected both at the mRNA and at the protein levels.

Expression of Tumor Treg Signature Genes is Negatively Correlated with Patients Survival

[0252] In an attempt to correlate our findings with clinical outcome, the inventors asked whether

the expression of the tumor-Treg signature transcripts correlated with disease prognosis in CRC and NSCLC patients. The inventors therefore interrogated for expression of Treg signature genes transcriptomic datasets obtained from resected tumor tissues of a cohort of 177 CRC patients (GSE17536 (Smith et al., 2010) and of a cohort of 263 NSCLC patients (GSE41271—(Sato et al., 2013), and correlated high and low gene expression levels with the 5-years survival data. Among those genes whose expression is highly enriched in tumor infiltrating Treg cells, LAYN, MAGEH1 and CCR8 were selected as they are the three genes more selectively expressed (FIG. **9**A-C). To normalize for differences in T cell densities within the resected tumor tissues, the inventors used the ratio between expression of the selected signature genes and CD3G. Remarkably, it was found that high expression of the three signature genes is in all cases correlated with a significantly reduced survival (FIG. **5**A). Interestingly, it was also observed that expressions of the three signature genes increased with tumor staging of CRC patients (FIG. **5**B).

[0253] In conclusion, high expression in the whole tumor samples of three genes (LAYN, MAGEH1 and CCR8) that are specifically and highly expressed in tumor infiltrating Treg cells, correlates with a poor prognosis in both NSCLC and CRC patients.

Selection of Potential Targets Specifically Over-Expressed on the Surface of Tumor-Infiltrating Treg

[0254] All annotated protein isoforms encoded by the 328 genes and retrievable in the public database EnsEMBL (http://www.ensembl.org) were simultaneously analysed with the four prediction algorithms and genes encoding at least one isoform predicted to be surface exposed were considered as potential targets.

[0255] Out of 328 genes, 193 encode for at least one potential cell surface protein isoform on the basis of at least one of the four predictors. The list of protein isoforms predicted to be membrane-associated is reported in Table VI.

TABLE-US-00010 TABLE VI SEQ ID No of the aa sequence of the Gene ENSG ID protein name Description release87 ENST ID ENSP ID isoform LAYN Layilin ENSG00000204381 ENST00000375614 ENSP00000364764 1 ENST00000375615 ENSP00000364765 2 ENST00000436913 ENSP00000392942 3 ENST00000525126 ENSP00000434328 4 ENST00000525866 ENSP00000434300 5 ENST00000528924 ENSP00000486561 6 ENST00000530962 ENSP00000431627 7 ENST00000533265 ENSP00000434972 8 ENST00000533999 ENSP00000432434 9 CCR8 C—C chemokine receptor ENSG00000179934 ENST00000326306 ENSP00000326432 10 type 8 ENST00000414803 ENSP00000390104 11 IL21R Interleukin-21 receptor ENSG00000103522 ENST00000337929 ENSP00000338010 12 ENST00000395754 ENSP00000379103 13 ENST00000564089 ENSP00000456707 14 FUCA2 Plasma alpha-L- ENSG00000001036 ENST00000002165 ENSP00000002165 15 fucosidase ENST00000451668 ENSP00000398119 16 ICA1 Islet cell autoantigen 1 ENSG00000003147 ENST00000407906 ENSP00000386021 17 COX10 Protoheme IX ENSG00000006695 ENST00000261643 ENSP00000261643 18 farnesyltransferase, mit. IL32 Interleukin-32 ENSG00000008517 ENST00000008180 ENSP00000008180 19 ENST00000396890 ENSP00000380099 20 ENST00000525228 ENSP00000431740 21 ENST00000525377 ENSP00000433866 22 ENST00000530890 ENSP00000433747 23 ENST00000534507 ENSP00000431775 24 ENST00000548246 ENSP00000447979 25 ENST00000548476 ENSP00000449483 26 ENST00000548807 ENSP00000448354 27 ENST00000551513 ENSP00000449147 28 ENST00000552356 ENSP00000446978 29 ENST00000552936 ENSP00000447033 30 ETV7 Transcription factor ENSG00000010030 ENST00000339796 ENSP00000342260 31 ETV7 ENST00000627426 ENSP00000486712 32 ATP2C1 Calcium-transporting ENSG00000017260 ENST00000328560 ENSP00000329664 33 ATPase type 2C member 1 ENST00000359644 ENSP00000352665 34 ENST00000422190 ENSP00000402677 35 ENST00000428331 ENSP00000395809 36 ENST00000504381 ENSP00000425320 37 ENST00000504571 ENSP00000422489 38 ENST00000504612 ENSP00000425228 39

ENST00000504948 ENSP00000423330 40 ENST00000505072 ENSP00000427625 41
ENST00000505330 ENSP00000423774 42 ENST00000507194 ENSP00000427087 43
ENST00000507488 ENSP00000421326 44 ENST00000508297 ENSP00000421261 45
ENST00000508532 ENSP00000424783 46 ENST00000508660 ENSP00000424930 47
ENST00000509662 ENSP00000426849 48 ENST00000510168 ENSP00000427461 49
ENST00000513801 ENSP00000422872 50 ENST00000515854 ENSP00000422890 51
ENST00000533801 ENSP00000432956 52 FAS Fatty acid synthase ENSG00000026103
ENST00000352159 ENSP00000345601 53 ENST00000355279 ENSP00000347426 54
ENST00000355740 ENSP00000347979 55 ENST00000357339 ENSP00000349896 56
ENST00000479522 ENSP00000424113 57 ENST00000484444 ENSP00000420975 58
ENST00000488877 ENSP00000425159 59 ENST00000492756 ENSP00000422453 60
ENST00000494410 ENSP00000423755 61 ENST00000612663 ENSP00000477997 62 PEX3
Peroxisomal biogenesis ENSG00000034693 ENST00000367591 ENSP00000356563 63 factor 3
ENST00000367592 ENSP00000356564 64 TSPAN17 Tetraspanin-17 ENSG00000048140
ENST00000298564 ENSP00000298564 65 ENST00000310032 ENSP00000309036 66
ENST00000503030 ENSP00000425975 67 ENST00000503045 ENSP00000425212 68
ENST00000504168 ENSP00000423957 69 ENST00000507471 ENSP00000423610 70
ENST00000508164 ENSP00000422053 71 ENST00000515708 ENSP00000426650 72 COL9A2
Collagen alpha-2(IX) ENSG00000049089 ENST00000372736 ENSP00000361821 73 chain
ENST00000372748 ENSP00000361834 74 ENST00000417105 ENSP00000388493 75 NFE2L3
Nuclear factor erythroid ENSG00000050344 ENST00000056233 ENSP00000056233 76 2-related
factor 3 TNIP3 TNFAIP3-interacting ENSG00000050730 ENST00000515036 ENSP00000424284
77 prot.3 LY75 Lymphocyte antigen 75 ENSG00000054219 ENST00000263636
ENSP00000263636 78 YIPF1 Protein YIPF1 ENSG00000058799 ENST00000072644
ENSP00000072644 79 ENST00000371399 ENSP00000360452 80 ENST00000412288
ENSP00000416507 81 ENST00000464950 ENSP00000432266 82 ISOC1 Isochorismatase
domain- ENSG00000066583 ENST00000173527 ENSP00000173527 83 containing protein 1
ENST00000514194 ENSP00000421273 84 ACSL4 Long-chain-fatty-acid-- ENSG00000068366
ENST00000340800 ENSP00000339787 85 CoA ligase 4 ENST00000469796 ENSP00000419171
86 ENST00000469857 ENSP00000423077 87 ENST00000502391 ENSP00000425408 88
ENST00000504980 ENSP00000421425 89 ENST00000508092 ENSP00000425378 90 MAST4
Microtubule-assoc.serine/ ENSG00000069020 ENST00000434115 ENSP00000396765 91
threonine-protein kinase 4 LMCD1 LIM and cysteine-rich ENSG00000071282 ENST00000456506
ENSP00000405049 92 domains protein 1 TFRC Transferrin receptor ENSG00000072274
ENST00000360110 ENSP00000353224 93 protein 1 ENST00000392396 ENSP00000376197 94
ENST00000421258 ENSP00000402839 95 ENST00000426789 ENSP00000414015 96 PANX2
Pannexin-2 ENSG00000073150 ENST00000159647 ENSP00000159647 97 ENST00000395842
ENSP00000379183 98 ENST00000402472 ENSP00000384148 99 FNDC3B Fibronectin type III
ENSG00000075420 ENST00000336824 ENSP00000338523 100 domain-containing
ENST00000415807 ENSP00000411242 101 protein 3B ENST00000416957 ENSP00000389094
102 ENST00000421757 ENSP00000408496 103 ENST00000423424 ENSP00000392471 104
IL12RB2 Interleukin-12 receptor ENSG00000081985 ENST00000262345 ENSP00000262345 105
subunit beta-2 ENST00000371000 ENSP00000360039 106 ENST00000441640
ENSP00000400959 107 ENST00000541374 ENSP00000445276 108 ENST00000544434
ENSP00000442443 109 STARD7 StAR-related lipid ENSG00000084090 ENST00000337288
ENSP00000338030 110 transfer protein 7, mitochondrial SSH1 Protein phosphatase
ENSG00000084112 ENST00000546697 ENSP00000446652 111 Slingshot homolog 1
ENST00000548522 ENSP00000448586 112 MGST2 Microsomal glutathione ENSG00000085871
ENST00000265498 ENSP00000265498 113 S-transferase 2 ENST00000503816
ENSP00000423008 114 ENST00000506797 ENSP00000424278 115 ENST00000616265

ENSP00000482639 116 ACOX3 Peroxisomal acyl- ENSG00000087008 ENST00000514423
ENSP00000427321 117 coenzyme A oxidase 3 ANKRD10 Ankyrin repeat domain-
ENSG00000088448 ENST00000603993 ENSP00000474638 118 containing protein 10 FKBP1A
Peptidyl-prolyl cis-trans ENSG00000088832 ENST00000612074 ENSP00000480846 119
isomerase FKBP1A ENST00000614856 ENSP00000482758 120 ENST00000618612
ENSP00000478093 121 SIRPG Signal-regulatory protein ENSG00000089012 ENST00000216927
ENSP00000216927 122 gamma ENST00000303415 ENSP00000305529 123 ENST00000344103
ENSP00000342759 124 ENST00000381580 ENSP00000370992 125 ENST00000381583
ENSP00000370995 126 WHRN Whirlin ENSG00000095397 ENST00000374059
ENSP00000363172 127 CENPM Centromere protein M ENSG00000100162 ENST00000215980
ENSP00000215980 128 ENST00000402338 ENSP00000384731 129 ENST00000402420
ENSP00000384132 130 ENST00000404067 ENSP00000384814 131 ENST00000407253
ENSP00000384743 132 NCF4 Neutrophil cytosol factor 4 ENSG00000100365 ENST00000447071
ENSP00000414958 133 CSF2RB Cytokine receptor ENSG00000100368 ENST00000262825
ENSP00000262825 134 common subunit beta ENST00000403662 ENSP00000384053 135
ENST00000406230 ENSP00000385271 136 ENST00000421539 ENSP00000393585 137 CNIH1
Protein cornichon ENSG00000100528 ENST00000216416 ENSP00000216416 138 homolog 1
ENST00000395573 ENSP00000378940 139 ENST00000553660 ENSP00000452457 140
ENST00000554683 ENSP00000452466 141 ENST00000556113 ENSP00000451142 142
ENST00000557659 ENSP00000451640 143 ENST00000557690 ENSP00000451852 144 PIGU
Phosphatidylinositol ENSG00000101464 ENST00000217446 ENSP00000217446 145 glycan
anchor ENST00000374820 ENSP00000363953 146 biosynthesis class U ENST00000438215
ENSP00000395755 147 protein NDFIP2 NEDD4 family- ENSG00000102471 ENST00000218652
ENSP00000218652 148 interacting protein 2 ENST00000487865 ENSP00000419200 149
ENST00000612570 ENSP00000480798 150 ENST00000620924 ENSP00000480881 151 ACP5
Tartrate-resistant acid ENSG00000102575 ENST00000218758 ENSP00000218758 152
phosphatase type 5 ENST00000412435 ENSP00000392374 153 ENST00000433365
ENSP00000413456 154 ENST00000589792 ENSP00000468685 155 ENST00000590420
ENSP00000468509 156 ENST00000590832 ENSP00000465127 157 ENST00000591319
ENSP00000464831 158 ENST00000592828 ENSP00000468767 159 NFAT5 Nuclear factor of
ENSG00000102908 ENST00000567990 ENSP00000455115 160 activated T-cells 5 CYB5B
Cytochrome b5 type B ENSG00000103018 ENST00000307892 ENSP00000308430 161
ENST00000512062 ENSP00000423679 162 ENST00000568237 ENSP00000464102 163
LAPTM4B Lysosomal-associated ENSG00000104341 ENST00000445593 ENSP00000402301
164 transmembrane protein 4B ENST00000517924 ENSP00000429868 165 ENST00000521545
ENSP00000428409 166 ENST00000619747 ENSP00000482533 167 IL7 Interleukin-7
ENSG00000104432 ENST00000263851 ENSP00000263851 168 ENST00000379113
ENSP00000368408 169 ENST00000518982 ENSP00000430272 170 ENST00000520215
ENSP00000428364 171 ENST00000520269 ENSP00000427750 172 ENST00000520317
ENSP00000427800 173 ENST00000541183 ENSP00000438922 174 EBI3 Interleukin-27 subunit
ENSG00000105246 ENST00000221847 ENSP00000221847 175 beta PLA2G4C Cytosolic
phospholipase ENSG00000105499 ENST00000595161 ENSP00000469528 176 A2 gamma
ENST00000595487 ENSP00000471328 177 ENST00000596352 ENSP00000471759 178
ENST00000598488 ENSP00000468972 179 GLCCI1 Glucocorticoid-induced ENSG00000106415
ENST00000430798 ENSP00000396171 180 transcript 1 protein MINPP1 Multiple inositol
ENSG00000107789 ENST00000371994 ENSP00000361062 181 polyphosphate
ENST00000371996 ENSP00000361064 182 phosphatase 1 ENST00000536010
ENSP00000437823 183 WSB1 WD repeat and SOCS ENSG00000109046 ENST00000581440
ENSP00000462737 184 box-containing protein 1 ENST00000582208 ENSP00000463621 185
ENST00000583193 ENSP00000462595 186 ENST00000583742 ENSP00000462365 187

HTATIP2 Oxidoreductase HTATIP2 ENSG00000109854 ENST00000419348 ENSP00000392985 188 ENST00000530266 ENSP00000436548 189 ENST00000532081 ENSP00000432107 190 ENST00000532505 ENSP00000432338 191 CTSC Dipeptidyl peptidase 1 ENSG00000109861 ENST00000227266 ENSP00000227266 192 ENST00000524463 ENSP00000432541 193 ENST00000527018 ENSP00000432556 194 ENST00000528020 ENSP00000433229 195 ENST00000529974 ENSP00000433539 196 VWA5A von Willebrand factor A ENSG00000110002 ENST00000392744 ENSP00000376501 197 domain-containing ENST00000392748 ENSP00000376504 198 protein 5A ENST00000456829 ENSP00000407726 199 SLC35F2 Solute carrier family 35 ENSG00000110660 ENST00000375682 ENSP00000364834 200 member F2 ENST00000525071 ENSP00000434307 201 ENST00000525815 ENSP00000436785 202 ENST00000532513 ENSP00000433783 203 VDR Vitamin D3 receptor ENSG00000111424 ENST00000547065 ENSP00000449074 204 SEC24A Protein transport protein ENSG00000113615 ENST00000398844 ENSP00000381823 205 Sec24A IL1R2 Interleukin-1 receptor ENSG00000115590 ENST00000332549 ENSP00000330959 206 type 2 ENST00000393414 ENSP00000377066 207 ENST00000441002 ENSP00000414611 208 ENST00000457817 ENSP00000408415 209 IL1R1 Interleukin-1 receptor ENSG00000115594 ENST00000409288 ENSP00000386478 210 type 1 ENST00000409329 ENSP00000387131 211 ENST00000409589 ENSP00000386555 212 ENST00000409929 ENSP00000386776 213 ENST00000410023 ENSP00000386380 214 ENST00000413623 ENSP00000407017 215 ENST00000422532 ENSP00000390349 216 ENST00000424272 ENSP00000415366 217 ENST00000428279 ENSP00000410461 218 ENST00000430171 ENSP00000408101 219 ENST00000442590 ENSP00000393296 220 ENST00000450319 ENSP00000411627 221 ENST00000452403 ENSP00000401646 222 IL1RL2 Interleukin-1 receptor- ENSG00000115598 ENST00000264257 ENSP00000264257 223 like 2 ENST00000421464 ENSP00000387611 224 ENST00000441515 ENSP00000413348 225 IL1RL1 Interleukin-1 receptor- ENSG00000115602 ENST00000233954 ENSP00000233954 226 like 1 ENST00000311734 ENSP00000310371 227 ENST00000404917 ENSP00000384822 228 ENST00000409584 ENSP00000386618 229 ENST00000427077 ENSP00000391120 230 ENST00000447231 ENSP00000409437 231 UXS1 UDP-glucuronic acid ENSG00000115652 ENST00000283148 ENSP00000283148 232 decarboxylase 1 ENST00000409501 ENSP00000387019 233 ENST00000441952 ENSP00000416656 234 ENST00000457835 ENSP00000399316 235 SLC25A12 Calcium-binding ENSG00000115840 ENST00000426896 ENSP00000413968 236 mitochondrial carrier protein Aralar1 THADA Thyroid adenoma- ENSG00000115970 ENST00000403856 ENSP00000385469 237 associated protein LEPR Leptin receptor ENSG00000116678 ENST00000344610 ENSP00000340884 238 ENST00000349533 ENSP00000330393 239 ENST00000371058 ENSP00000360097 240 ENST00000371059 ENSP00000360098 241 ENST00000371060 ENSP00000360099 242 ENST00000406510 ENSP00000384025 243 ENST00000616738 ENSP00000483390 244 MREG Melanoregulin ENSG00000118242 ENST00000263268 ENSP00000263268 245 ENST00000620139 ENSP00000484331 246 FLVCR2 Feline leukemia virus ENSG00000119686 ENST00000238667 ENSP00000238667 247 subgroup C receptor- ENST00000539311 ENSP00000443439 248 related protein 2 ENST00000553341 ENSP00000452584 249 ENST00000553587 ENSP00000451603 250 ENST00000554580 ENSP00000451781 251 ENST00000555027 ENSP00000452453 252 ENST00000555058 ENSP00000451104 253 ENST00000556856 ENSP00000452468 254 SOCS2 Suppressor of cytokine ENSG00000120833 ENST00000548537 ENSP00000448709 255 signaling 2 ENST00000549510 ENSP00000474888 256 RDH10 Retinol dehydrogenase 10 ENSG00000121039 ENST00000240285 ENSP00000240285 257 ENST00000519380 ENSP00000428132 258 ENST00000521928 ENSP00000429727 259 LAX1 Lymphocyte ENSG00000122188 ENST00000367217 ENSP00000356186 260 transmembrane adapter 1 ENST00000442561 ENSP00000406970 261 ZWINT ZW10 interactor ENSG00000122952 ENST00000489649 ENSP00000473330 262 ACOT9

Acyl-coenzyme A ENSG00000123130 ENST00000336430 ENSP00000336580 263 thioesterase 9, ENST00000379303 ENSP00000368605 264 mitochondrial ENST00000494361 ENSP00000420238 265 TM9SF2 Transmembrane 9 ENSG00000125304 ENST00000376387 ENSP00000365567 266 superfamily member 2 HS3ST3B1 Heparan sulfate ENSG00000125430 ENST00000360954 ENSP00000354213 267 glucosamine 3-O- ENST00000466596 ENSP00000436078 268 sulfotransferase 3B1 EML2 Echinoderm ENSG00000125746 ENST00000245925 ENSP00000245925 269 microtubule-associated ENST00000586195 ENSP00000465339 270 protein-like 2 ENST00000586405 ENSP00000465885 271 ENST00000586770 ENSP00000465786 272 ENST00000587152 ENSP00000468312 273 ENST00000587484 ENSP00000465994 274 ENST00000588272 ENSP00000466100 275 ENST00000588308 ENSP00000468329 276 ENST00000589876 ENSP00000464789 277 ENST00000590018 ENSP00000468373 278 ENST00000590043 ENSP00000464804 279 ENST00000590819 ENSP00000464950 280 ENST00000591721 ENSP00000468470 281 ENST00000592853 ENSP00000468383 282 ENST00000593255 ENSP00000467941 283 MGME1 Mitochondrial genome ENSG00000125871 ENST00000377704 ENSP00000366933 284 maintenance ENST00000377709 ENSP00000366938 285 exonuclease 1 ENST00000377710 ENSP00000366939 286 IGFLR1 IGF-like family receptor 1 ENSG00000126246 ENST00000246532 ENSP00000246532 287 ENST00000588018 ENSP00000468545 288 ENST00000588992 ENSP00000465962 289 ENST00000591277 ENSP00000468644 290 ENST00000591748 ENSP00000476009 291 ENST00000592537 ENSP00000466181 292 ENST00000592693 ENSP00000474913 293 ENST00000592889 ENSP00000467750 294 MYO5C Unconventional myosin- ENSG00000128833 ENST00000261839 ENSP00000261839 295 Vc ITFG1 T-cell ENSG00000129636 ENST00000320640 ENSP00000319918 296 immunomodulatory ENST00000544001 ENSP00000441062 297 protein ENST00000563730 ENSP00000455630 298 ENST00000565262 ENSP00000457665 299 ENST00000565940 ENSP00000459192 300 SYT11 Synaptotagmin-11 ENSG00000132718 ENST00000368324 ENSP00000357307 301 SLC41A1 Solute carrier family 41 ENSG00000133065 ENST00000367137 ENSP00000356105 302 member 1 ATP13A3 Probable cation- ENSG00000133657 ENST00000256031 ENSP00000256031 303 transporting ATPase ENST00000429136 ENSP00000402550 304 13A3 ENST00000439040 ENSP00000416508 305 ENST00000446356 ENSP00000410767 306 ENST00000457986 ENSP00000406234 307 ENST00000619199 ENSP00000482200 308 MICAL2 Protein-methionine ENSG00000133816 ENST00000379612 ENSP00000368932 309 sulfoxide oxidase MICAL2 CABLES1 CDK5 and ABL1 enzyme ENSG00000134508 ENST00000256925 ENSP00000256925 310 substrate 1 ENST00000579963 ENSP00000464435 311 HAVCR2 Hepatitis A virus cellular ENSG00000135077 ENST00000307851 ENSP00000312002 312 receptor 2 ENST00000522593 ENSP00000430873 313 CGA Chromogranin-A ENSG00000135346 ENST00000369582 ENSP00000358595 314 ENST00000610310 ENSP00000482232 315 ENST00000625577 ENSP00000486666 316 ENST00000627148 ENSP00000486024 317 ENST00000630630 ENSP00000487300 318 FAIM2 Protein lifeguard 2 ENSG00000135472 ENST00000320634 ENSP00000321951 319 ENST00000547871 ENSP00000449360 320 ENST00000550195 ENSP00000447715 321 ENST00000550635 ENSP00000449711 322 ENST00000550890 ENSP00000450132 323 ENST00000552669 ENSP00000446771 324 ENST00000552863 ENSP00000449957 325 ARHGEF4 Rho guanine nucleotide ENSG00000136002 ENST00000392953 ENSP00000376680 326 exchange factor 4 SLC41A2 Solute carrier family 41 ENSG00000136052 ENST00000258538 ENSP00000258538 327 member 2 ENST00000437220 ENSP00000391377 328 NUSAP1 Nucleolar and spindle- ENSG00000137804 ENST00000557840 ENSP00000453428 329 associated protein 1 ENST00000559046 ENSP00000452725 330 ADAM10 Disintegrin and ENSG00000137845 ENST00000260408 ENSP00000260408 331 metalloproteinase ENST00000396136 ENSP00000456542 332 domain-containing ENST00000402627 ENSP00000386056 333 protein 10 ENST00000439637 ENSP00000391930

334 ENST00000461408 ENSP00000481779 335 ENST00000558004 ENSP00000452704 336 ENST00000559053 ENSP00000453952 337 ENST00000561288 ENSP00000452639 338 HADHB Trifunctional enzyme ENSG00000138029 ENST00000545822 ENSP00000442665 339 subunit beta, mitochondrial CD27 CD27 antigen ENSG00000139193 ENST00000266557 ENSP00000266557 340 CDH24 Cadherin-24 ENSG00000139880 ENST00000267383 ENSP00000267383 341 ENST00000397359 ENSP00000380517 342 ENST00000487137 ENSP00000434821 343 ENST00000554034 ENSP00000452493 344 ENST00000610348 ENSP00000478078 345 ETFA Electron transfer ENSG00000140374 ENST00000560044 ENSP00000452942 346 alpha, mitochondrial ENST00000560309 ENSP00000453753 347 flavoprotein subunit KSR1 Kinase suppressor of Ras 1 ENSG00000141068 ENST00000580163 ENSP00000463204 348 SECTM1 Secreted and ENSG00000141574 ENST00000269389 ENSP00000269389 349 transmembrane protein 1 ENST00000580437 ENSP00000463904 350 ENST00000581691 ENSP00000463114 351 ENST00000581864 ENSP00000464111 352 ENST00000581954 ENSP00000464385 353 ENST00000582290 ENSP00000462294 354 ENST00000582563 ENSP00000463120 355 ENST00000583093 ENSP00000462563 356 EVA1B Protein eva-1 homolog B ENSG00000142694 ENST00000270824 ENSP00000270824 357 CTTNBP2NL CTTNBP2 N-terminal-like ENSG00000143079 ENST00000271277 ENSP00000271277 358 protein ENST00000441739 ENSP00000390976 359 CASQ1 Calsequestrin-1 ENSG00000143318 ENST00000368078 ENSP00000357057 360 ARL6IP5 PRA1 family protein 3 ENSG00000144746 ENST00000273258 ENSP00000273258 361 ENST00000478935 ENSP00000420138 362 ENST00000484921 ENSP00000419374 363 ENST00000485444 ENSP00000419021 364 ADPRH [Protein ADP- ENSG00000144843 ENST00000357003 ENSP00000349496 365 ribosylarginine] ENST00000465513 ENSP00000417430 366 hydrolase ENST00000478399 ENSP00000420200 367 ENST00000478927 ENSP00000417528 368 ENST00000481816 ENSP00000419703 369 PAM Peptidyl-glycine alpha- ENSG00000145730 ENST00000304400 ENSP00000306100 370 amidating ENST00000345721 ENSP00000302544 371 monooxygenase ENST00000346918 ENSP00000282992 372 ENST00000348126 ENSP00000314638 373 ENST00000438793 ENSP00000396493 374 ENST00000455264 ENSP00000403461 375 ENST00000504691 ENSP00000424203 376 ENST00000505654 ENSP00000421569 377 ENST00000506006 ENSP00000423611 378 ENST00000509832 ENSP00000423763 379 ENST00000511477 ENSP00000421823 380 ENST00000511839 ENSP00000426448 381 ENST00000512073 ENSP00000420851 382 RNF145 RING finger protein 145 ENSG00000145860 ENST00000274542 ENSP00000274542 383 ENST00000424310 ENSP00000409064 384 ENST00000518802 ENSP00000430955 385 ENST00000519865 ENSP00000430397 386 ENST00000520638 ENSP00000429071 387 ENST00000521606 ENSP00000430753 388 ENST00000611185 ENSP00000482720 389 TMEM140 Transmembrane protein ENSG00000146859 ENST00000275767 ENSP00000275767 390 140 CHST7 Carbohydrate ENSG00000147119 ENST00000276055 ENSP00000276055 391 sulfotransferase 7 CHRNA6 Neuronal acetylcholine ENSG00000147434 ENST00000276410 ENSP00000276410 392 receptor subunit alpha-6 ENST00000533810 ENSP00000434659 393 ENST00000534622 ENSP00000433871 394 PTPRJ Receptor-type tyrosine- ENSG00000149177 ENST00000418331 ENSP00000400010 395 protein phosphatase eta ENST00000440289 ENSP00000409733 396 ENST00000527952 ENSP00000435618 397 ENST00000534219 ENSP00000432686 398 ENST00000613246 ENSP00000477933 399 ENST00000615445 ENSP00000479342 400 NCAM1 Neural cell adhesion ENSG00000149294 ENST00000316851 ENSP00000318472 401 molecule 1 ENST00000401611 ENSP00000384055 402 ENST00000524916 ENSP00000478072 403 ENST00000526322 ENSP00000479687 404 ENST00000528158 ENSP00000486241 405 ENST00000528590 ENSP00000480269 406 ENST00000529356 ENSP00000482205 407 ENST00000531044 ENSP00000484943 408 ENST00000531817 ENSP00000475074 409

ENST00000533073 ENSP00000486406 410 ENST00000613217 ENSP00000479353 411
ENST00000615112 ENSP00000480797 412 ENST00000615285 ENSP00000479241 413
ENST00000618266 ENSP00000477835 414 ENST00000619839 ENSP00000480132 415
ENST00000620046 ENSP00000482852 416 ENST00000621128 ENSP00000481083 417
ENST00000621518 ENSP00000477808 418 ENST00000621850 ENSP00000480774 419 INPP1
Inositol polyphosphate ENSG00000151689 ENST00000413239 ENSP00000391415 420 1-
phosphatase ENST00000444194 ENSP00000404732 421 ENST00000451089 ENSP00000410662
422 ENST00000458193 ENSP00000412119 423 JAKMIP1 Janus kinase and ENSG00000152969
ENST00000409021 ENSP00000386711 424 protein 1 ENST00000409371 ENSP00000387042 425
microtubule-interacting RHOC Rho-related GTP-binding ENSG00000155366 ENST00000468093
ENSP00000431392 426 protein RhoC ENST00000484280 ENSP00000434310 427
ENST00000528831 ENSP00000432209 428 SLC16A1 Monocarboxylate ENSG00000155380
ENST00000369626 ENSP00000358640 429 transporter 1 ENST00000429288 ENSP00000397106
430 ENST00000443580 ENSP00000399104 431 ENST00000458229 ENSP00000416167 432
ENST00000538576 ENSP00000441065 433 CXCL13 C—X—C motif chemokine
ENSG00000156234 ENST00000286758 ENSP00000286758 434 13 SH3RF2 Putative E3
ubiquitin- ENSG00000156463 ENST00000359120 ENSP00000352028 435 protein ligase SH3RF2
ENST00000511217 ENSP00000424497 436 NPTN Neuroplastin ENSG00000156642
ENST00000345330 ENSP00000290401 437 ENST00000351217 ENSP00000342958 438
ENST00000562924 ENSP00000456349 439 ENST00000563691 ENSP00000457028 440
ENST00000565325 ENSP00000457470 441 AHCYL2 Adenosylhomocysteinase 3
ENSG00000158467 ENST00000466924 ENSP00000419346 442 PTGIR Prostacyclin receptor
ENSG00000160013 ENST00000291294 ENSP00000291294 443 ENST00000594275
ENSP00000469408 444 ENST00000596260 ENSP00000468970 445 ENST00000597185
ENSP00000470566 446 ENST00000598865 ENSP00000470799 447 TMPRSS3 Transmembrane
ENSG00000160183 ENST00000291532 ENSP00000291532 448 protease serine 4
ENST00000398397 ENSP00000381434 449 ENST00000398405 ENSP00000381442 450
ENST00000433957 ENSP00000411013 451 FCRL3 Fc receptor-like protein 3 ENSG00000160856
ENST00000368184 ENSP00000357167 452 ENST00000368186 ENSP00000357169 453
ENST00000477837 ENSP00000433430 454 ENST00000485028 ENSP00000434331 455
ENST00000492769 ENSP00000435487 456 ENST00000496769 ENSP00000473680 457 PAQR4
Progestin and adipoQ ENSG00000162073 ENST00000293978 ENSP00000293978 458 receptor
family member 4 ENST00000318782 ENSP00000321804 459 ENST00000572687
ENSP00000459418 460 ENST00000574988 ENSP00000458683 461 ENST00000576565
ENSP00000460326 462 ZG16B Zymogen granule ENSG00000162078 ENST00000382280
ENSP00000371715 463 protein 16 homolog B ENST00000570670 ENSP00000460793 464
ENST00000571723 ENSP00000458847 465 ENST00000572863 ENSP00000461740 466 SGPP2
Sphingosine-1- ENSG00000163082 ENST00000321276 ENSP00000315137 467 phosphate
phosphatase 2 NEURL3 E3 ubiquitin-protein ENSG00000163121 ENST00000310865
ENSP00000479456 468 ligase NEURL1B ENST00000435380 ENSP00000480933 469 KIF15
Kinesin-like protein ENSG00000163808 ENST00000438321 ENSP00000406939 470 KIF15
TMEM184C Transmembrane protein ENSG00000164168 ENST00000296582 ENSP00000296582
471 184C ENST00000505999 ENSP00000421159 472 ENST00000508208 ENSP00000425940
473 C5ORF63 Glutaredoxin-like protein ENSG00000164241 ENST00000296662
ENSP00000453964 474 C5orf63 ENST00000508527 ENSP00000475157 475 ENST00000509733
ENSP00000475415 476 ENST00000535381 ENSP00000454153 477 ENST00000606042
ENSP00000475733 478 ENST00000606937 ENSP00000475810 479 ENST00000607731
ENSP00000476160 480 MELK Maternal embryonic ENSG00000165304 ENST00000495529
ENSP00000487536 481 leucine zipper kinase ENST00000536329 ENSP00000443550 482
ENST00000536987 ENSP00000439184 483 ENST00000543751 ENSP00000441596 484

ENST00000626154 ENSP00000486558 485 FAAH2 Fatty-acid amide ENSG00000165591
ENST00000374900 ENSP00000364035 486 hydrolase 2 TPP1 Alpha-tocopherol
ENSG00000166340 ENST00000299427 ENSP00000299427 487 transfer protein
ENST00000436873 ENSP00000398136 488 ENST00000528571 ENSP00000434647 489
ENST00000528657 ENSP00000435001 490 CX3CR1 CX3C chemokine ENSG00000168329
ENST00000358309 ENSP00000351059 491 receptor 1 ENST00000399220 ENSP00000382166
492 ENST00000412814 ENSP00000408835 493 ENST00000435290 ENSP00000394960 494
ENST00000541347 ENSP00000439140 495 ENST00000542107 ENSP00000444928 496 TSPAN5
Tetraspanin-5 ENSG00000168785 ENST00000305798 ENSP00000307701 497
ENST00000505184 ENSP00000423916 498 ENST00000508798 ENSP00000421808 499
ENST00000511651 ENSP00000426248 500 ENST00000511800 ENSP00000422548 501
ENST00000515287 ENSP00000423504 502 ENST00000515440 ENSP00000422351 503 UGP2
UTP--glucose-1- ENSG00000169764 ENST00000467999 ENSP00000418642 504
uridylyltransferase ENST00000496334 ENSP00000420760 505 phosphate GLB1 Beta-
galactosidase ENSG00000170266 ENST00000307363 ENSP00000306920 506
ENST00000307377 ENSP00000305920 507 ENST00000399402 ENSP00000382333 508
ENST00000415454 ENSP00000411813 509 ENST00000436768 ENSP00000387989 510
ENST00000438227 ENSP00000401250 511 ENST00000440656 ENSP00000411769 512
ENST00000446732 ENSP00000407365 513 ENST00000450835 ENSP00000403264 514
SPATA24 Spermatogenesis- ENSG00000170469 ENST00000514983 ENSP00000423424 515
associated protein 24 RBKS Ribokinase ENSG00000171174 ENST00000449378
ENSP00000413789 516 NETO2 Neuropilin and tolloid- ENSG00000171208 ENST00000303155
ENSP00000306726 517 like protein 2 ENST00000562435 ENSP00000455169 518
ENST00000562559 ENSP00000454213 519 ENST00000563078 ENSP00000456818 520
ENST00000564667 ENSP00000457133 521 LRG1 Leucine-rich alpha-2- ENSG00000171236
ENST00000306390 ENSP00000302621 522 glycoprotein FAM98B Protein FAM98B
ENSG00000171262 ENST00000491535 ENSP00000453166 523 ENST00000559431
ENSP00000453926 524 CHST11 Carbohydrate ENSG00000171310 ENST00000303694
ENSP00000305725 525 sulfotransferase 11 ENST00000546689 ENSP00000448678 526
ENST00000547956 ENSP00000449093 527 ENST00000549260 ENSP00000450004 528 ECEL1
Endothelin-converting ENSG00000171551 ENST00000304546 ENSP00000302051 529 enzyme-
like 1 ENST00000409941 ENSP00000386333 530 BCL2L1 Bcl-2-like protein 1
ENSG00000171552 ENST00000307677 ENSP00000302564 531 ENST00000376055
ENSP00000365223 532 ENST00000376062 ENSP00000365230 533 MALT1 Mucosa-associated
ENSG00000172175 ENST00000345724 ENSP00000304161 534 lymphoid tissue
ENST00000348428 ENSP00000319279 535 lymphoma translocation ENST00000591792
ENSP00000467222 536 protein 1 CYP7B1 25-hydroxycholesterol 7- ENSG00000172817
ENST00000310193 ENSP00000310721 537 alpha-hydroxylase HPSE Heparanase
ENSG00000173083 ENST00000311412 ENSP00000308107 538 ENST00000405413
ENSP00000384262 539 ENST00000507150 ENSP00000426139 540 ENST00000508891
ENSP00000421827 541 ENST00000509906 ENSP00000421038 542 ENST00000512196
ENSP00000423265 543 ENST00000513463 ENSP00000421365 544 VANGL1 Vang-like protein 1
ENSG00000173218 ENST00000310260 ENSP00000310800 545 ENST00000355485
ENSP00000347672 546 ENST00000369509 ENSP00000358522 547 ENST00000369510
ENSP00000358523 548 CD7 T-cell antigen CD7 ENSG00000173762 ENST00000312648
ENSP00000312027 549 ENST00000578509 ENSP00000464565 550 ENST00000581434
ENSP00000464546 551 ENST00000582480 ENSP00000464182 552 ENST00000583376
ENSP00000463489 553 ENST00000584284 ENSP00000463612 554 HAP1 Huntingtin-associated
ENSG00000173805 ENST00000455021 ENSP00000397242 555 protein 1 FBXO45 F-box/SPRY
domain- ENSG00000174013 ENST00000440469 ENSP00000389868 556 containing protein 1

CHST2 Carbohydrate ENSG00000175040 ENST00000309575 ENSP00000307911 557
sulfotransferase 2 RM12 RecQ-mediated genome ENSG00000175643 ENST00000572173
ENSP00000461206 558 instability protein 2 SLC35E3 Solute carrier family 35 ENSG00000175782
ENST00000398004 ENSP00000381089 559 member E3 ENST00000431174 ENSP00000403769
560 ZBTB38 Zinc finger and BTB ENSG00000177311 ENST00000503809 ENSP00000422051
561 domain-containing protein 38 YIPF6 Protein YIPF6 ENSG00000181704 ENST00000374622
ENSP00000363751 562 ENST00000451537 ENSP00000401799 563 ENST00000462683
ENSP00000417573 564 CREB3L2 Cyclic AMP-responsive ENSG00000182158
ENST00000330387 ENSP00000329140 565 element-binding protein ENST00000420629
ENSP00000402889 566 3-like protein 2 ENST00000456390 ENSP00000403550 567 XKRX XK-
related protein 2 ENSG00000182489 ENST00000372956 ENSP00000362047 568
ENST00000468904 ENSP00000419884 569 CADM1 Cell adhesion molecule 1
ENSG00000182985 ENST00000331581 ENSP00000329797 570 ENST00000452722
ENSP00000395359 571 ENST00000536727 ENSP00000440322 572 ENST00000537058
ENSP00000439817 573 ENST00000540951 ENSP00000445375 574 ENST00000542447
ENSP00000439176 575 ENST00000542450 ENSP00000442001 576 ENST00000543540
ENSP00000439847 577 ENST00000545380 ENSP00000442387 578 ENST00000612235
ENSP00000483648 579 ENST00000612471 ENSP00000483793 580 ENST00000616271
ENSP00000484516 581 ENST00000621043 ENSP00000482840 582 ENST00000621709
ENSP00000482924 583 LHFP Lipoma HMGIC fusion ENSG00000183722 ENST00000379589
ENSP00000368908 584 partner CSF1 Macrophage colony- ENSG00000184371
ENST00000329608 ENSP00000327513 585 stimulating factor 1 ENST00000357302
ENSP00000349854 586 ENST00000369801 ENSP00000358816 587 ENST00000369802
ENSP00000358817 588 ENST00000420111 ENSP00000407317 589 ENST00000488198
ENSP00000433837 590 ENST00000525659 ENSP00000431547 591 ENST00000527192
ENSP00000434527 592 PTP4A3 Protein tyrosine ENSG00000184489 ENST00000329397
ENSP00000332274 593 phosphatase type IVA 3 ENST00000349124 ENSP00000331730 594
ENST00000520105 ENSP00000428758 595 ENST00000521578 ENSP00000428976 596
ENST00000523147 ENSP00000428725 597 ENST00000524028 ENSP00000430332 598 OSBP2
Oxysterol-binding ENSG00000184792 ENST00000445781 ENSP00000411497 599 protein 2
METTL7A Methyltransferase-like ENSG00000185432 ENST00000332160 ENSP00000331787
600 protein 7A ENST00000547104 ENSP00000447542 601 ENST00000548553
ENSP00000448785 602 ENST00000550097 ENSP00000448286 603 ENST00000550502
ENSP00000450239 604 TMPRSS6 Transmembrane ENSG00000187045 ENST00000346753
ENSP00000334962 605 protease serine 6 ENST00000381792 ENSP00000371211 606
ENST00000406725 ENSP00000385453 607 ENST00000406856 ENSP00000384964 608
ENST00000423761 ENSP00000400317 609 ENST00000429068 ENSP00000392433 610
ENST00000442782 ENSP00000397691 611 GCNT1 Beta-1,3-galactosyl-O- ENSG00000187210
ENST00000376730 ENSP00000365920 612 glycosyl-glycoprotein ENST00000442371
ENSP00000415454 613 beta-1,6-N- ENST00000444201 ENSP00000390703 614
acetylglucosaminyltransferase MAGEH1 Melanoma-associated ENSG00000187601
ENST00000342972 ENSP00000343706 615 antigen H1 NEMP2 Nuclear envelope
ENSG00000189362 ENST00000343105 ENSP00000340087 616 integral membrane
ENST00000409150 ENSP00000386292 617 protein 2 ENST00000414176 ENSP00000404283 618
ENST00000421038 ENSP00000410306 619 ENST00000444545 ENSP00000403867 620 NTNG2
Netrin-G2 ENSG00000196358 ENST00000372179 ENSP00000361252 621 ENST00000393229
ENSP00000376921 622 PDGFA Platelet-derived growth ENSG00000197461 ENST00000354513
ENSP00000346508 623 factor subunit A ENST00000400761 ENSP00000383572 624
ENST00000402802 ENSP00000383889 625 ENST00000405692 ENSP00000384673 626
PDCD1LG2 Programmed cell death 1 ENSG00000197646 ENST00000397747 ENSP00000380855

627 ligand 2 TOR4A Torsin-4A ENSG00000198113 ENST00000357503 ENSP00000350102 628 HIBCH 3-hydroxyisobutyryl-CoA ENSG00000198130 ENST00000392333 ENSP00000376145 629 hydrolase, mitochondrial ENST00000414928 ENSP00000414820 630 NTRK1 High affinity nerve ENSG00000198400 ENST00000358660 ENSP00000351486 631 growth factor receptor ENST00000368196 ENSP00000357179 632 ENST00000392302 ENSP00000376120 633 ENST00000497019 ENSP00000436804 634 ENST00000524377 ENSP00000431418 635 FAM19A2 Protein FAM19A2 ENSG00000198673 ENST00000416284 ENSP00000393987 636 ENST00000548780 ENSP00000449310 637 ENST00000549379 ENSP00000447584 638 ENST00000549958 ENSP00000447280 639 ENST00000550003 ENSP00000449457 640 ENST00000551449 ENSP00000449632 641 ENST00000551619 ENSP00000447305 642 ENST00000552075 ENSP00000449516 643 F5 Coagulation factor V ENSG00000198734 ENST00000367796 ENSP00000356770 644 ENST00000367797 ENSP00000356771 645 GK Glycerol kinase ENSG00000198814 ENST00000378943 ENSP00000368226 646 ENST00000427190 ENSP00000401720 647 ENST00000488296 ENSP00000419771 648 INPP5F Phosphatidylinositide ENSG00000198825 ENST00000490818 ENSP00000487706 649 phosphatase SAC2 ENST00000631572 ENSP00000488726 650 CD177 CD177 antigen ENSG00000204936 ENST00000378012 ENSP00000367251 651 ENST00000607855 ENSP00000483817 652 ENST00000618265 ENSP00000479536 653 LEPROT Leptin Receptor ENSG00000213625 ENST00000371065 ENSP00000360104 654 Overlapping Transcrip ENST00000613538 ENSP00000483521 655 TRIM16 Tripartite motif- ENSG00000221926 ENST00000579219 ENSP00000463639 656 containing protein 16 LTA Lymphotoxin-alpha ENSG00000226979 ENST00000418386 ENSP00000413450 657 ENST00000454783 ENSP00000403495 658 PROB1 Proline-rich basic protein 1 ENSG00000228672 ENST00000434752 ENSP00000416033 659 SSTR3 Somatostatin receptor ENSG00000278195 ENST00000610913 ENSP00000480971 660 type 3 ENST00000617123 ENSP00000481325 661 CEACAM1 Carcinoembryonic ENSG00000079385 ENST00000161559 ENSP00000161559 662 antigen-related cell ENST00000352591 ENSP00000244291 663 adhesion molecule 1 ENST00000358394 ENSP00000351165 664 ENST00000403444 ENSP00000384709 665 ENST00000403461 ENSP00000384083 666 ENST00000471298 ENSP00000472633 667 ENST00000599389 ENSP00000471918 668 ENST00000600172 ENSP00000471566 669 CTLA4 Cytotoxic T-lymphocyte ENSG00000163599 ENST00000295854 ENSP00000295854 670 protein 4 ENST00000302823 ENSP00000303939 671 ENST00000427473 ENSP00000409707 672 ENST00000472206 ENSP00000417779 673 TIGIT T-cell immunoreceptor ENSG00000181847 ENST00000383671 ENSP00000373167 674 with Ig and ITIM ENST00000461158 ENSP00000418917 675 domains ENST00000481065 ENSP00000420552 676 ENST00000484319 ENSP00000419706 677 ENST00000486257 ENSP00000419085 678 IL2RA Interleukin-2 receptor ENSG00000134460 ENST00000256876 ENSP00000256876 679 subunit alpha ENST00000379954 ENSP00000369287 680 ENST00000379959 ENSP00000369293 681 ENTPD1 Ectonucleoside ENSG00000138185 ENST00000371205 ENSP00000360248 682 triphosphate ENST00000371207 ENSP00000360250 683 diphosphohydrolase 1 ENST00000453258 ENSP00000390955 684 ENST00000483213 ENSP00000489333 685 ENST00000543964 ENSP00000442968 686 ENST00000635076 ENSP00000489250 687 ICOS Inducible T-cell ENSG00000163600 ENST00000316386 ENSP00000319476 688 costimulator ENST00000435193 ENSP00000415951 689 TNFRSF4 Tumor necrosis factor ENSG00000186827 ENST00000379236 ENSP00000368538 690 receptor superfamily member 4 TNFRSF18 Tumor necrosis factor ENSG00000186891 ENST00000328596 ENSP00000328207 691 receptor superfamily ENST00000379265 ENSP00000368567 692 member 18 ENST00000379268 ENSP00000368570 693 ENST00000486728 ENSP00000462735 694 TNFRSF8 Tumor necrosis factor ENSG00000120949 ENST00000263932 ENSP00000263932 695 receptor superfamily ENST00000417814 ENSP00000390650 696 member 8 ENST00000514649 ENSP00000421938

697 CD274 Programmed cell death 1 ENSG00000120217 ENST00000381573 ENSP00000370985
698 ligand 1 ENST00000381577 ENSP00000370989 699 IL2RB Interleukin-2 receptor
ENSG00000100385 ENST00000216223 ENSP00000216223 700 subunit beta ENST00000429622
ENSP00000402685 701 ENST00000445595 ENSP00000401020 702 ENST00000453962
ENSP00000403731 703 TNFRSF9 Tumor necrosis factor ENSG00000049249 ENST00000377507
ENSP00000366729 704 receptor superfamily ENST00000474475 ENSP00000465272 705 member
9 ENST00000615230 ENSP00000478699 706 IKZF2 Zinc finger protein Helios
ENSG00000030419 ENST00000442445 ENSP00000390045 707

[0256] Genes of table VI are characterized by their Ensembl Gene accession number (ENSG), retrievable in the public database EnsEMBL (http://www.ensembl.org). Each related protein isoform is characterized by an Ensembl transcript accession number (ENST) and an Ensembl protein accession number (ENSP).

Identification of Transcript Isoforms Expressed by Tumor-Treg Cells

[0257] An important aspect to be verified in the selection of potential targets of tumor-T reg is that the protein isoforms predicted to be surface exposed/membrane associated by the cell localization algorithms are indeed expressed in tumor Treg cells. Thus, total RNA was extracted from tumor Treg cells isolated from NSCLC or CRC samples and subjected to RT-PCR using specific primer pairs able to discriminate the different isoforms annotated for each gene. Exemplificative results of protein isoforms predicted to be surface exposed and detected in tumor T reg cells is reported in Table VII. Moreover, an example of RT-PCR analysis carried out for SIRPG is reported in FIG. **10**.

TABLE-US-00011 TABLE VII Representative examples of transcripts detected in tumor-infiltrating Treg cells GENE SYMBOL Surface predicted isoform detected in Tumor Treg cells CCR8
ENST00000326306 LAYN ENST00000375614 and/or ENST00000533265 and/or
ENST00000375615 and/or ENST00000525126 CD7 ENST00000312648 and/or
ENST00000584284 CXCL13 ENST00000286758 FCRL3 ENST00000492769 and/or
ENST00000368184 and/or ENST00000368186 and/or ENST00000485028 IL1R2
ENST00000332549 and/or ENST00000393414 IL21R ENST00000337929 and/or
ENST00000395754 and/or ENST00000564089 NTNG2 ENST00000393229 SIRPG
ENST00000303415 and/or ENST00000216927 and/or ENST00000344103 and/or
ENST00000381580 and/or ENST00000381583 TSPAN5 ENST00000305798 and/or
ENST00000505184 TMPRSS3 ENST00000291532 TMPRSS6 ENST00000406725 and/or
ENST00000406856 NDFIP2 ENST00000218652

Discussion

[0258] Diversity of tumor infiltrating Treg cells should be fully elucidated to understand their functional relevance and prognostic significance in different types of cancer, and to possibly improve the therapeutic efficacy of Treg cell modulation through the selective depletion of tumor infiltrating Treg cells. The transcriptome analysis performed on CRC- and NSCLC-infiltrating T cells showed that tumor-infiltrating Treg cells are different from both circulating and normal tissue-infiltrating Tregs, suggesting that the tumor microenvironment influences specific gene expression in Treg cells. Our findings further support the view that Treg cells from different tissues are instructed by environmental factors to display different gene expression profiles (Panduro et al., 2016). Indeed the list of signature genes includes a number of molecules that are consistently upregulated in tumor infiltrating Treg cells isolated from different tumor types, and these signature genes would have not been identified if the inventors had not profiled specifically tumor infiltrating Treg cells. It was found tumor-infiltrating-Treg signature genes are not only largely shared between CRC and NSCLC infiltrating cells, but are also conserved in breast and gastric cancers as well as in CRC and NSCLC metastatic tumors (in liver and brain respectively) suggesting that expression of these genes is a common feature of tumor infiltrating Treg cells that may correlate with Treg cells specific function within the tumor microenvironment. Although our knowledge on the function of immune checkpoints on lymphocytes is still incomplete, agonist or antagonist monoclonal

antibodies targeting checkpoints are in clinical development. Interestingly, it has been found that some of these checkpoints (such as GITR, OX40, TIGIT, LAG-3 and TIM-3) and some of their ligands (such as OX40LG, Galectin-9, CD70) are upregulated also in tumor infiltrating Treg cells, and this fact should be taken into account in interpreting clinical results with checkpoint inhibitors. Indeed, it is likely that assessment of the expression of checkpoints and of their ligands on the various subsets of tumor infiltrating lymphocytes will help to elucidate conflicting results and provide the rationale for combination therapies. Therefore, expression pattern of checkpoints should be evaluated both in tumor infiltrating lymphocytes and in tumor cells. Single-cell analysis on selected tumor Treg signature genes confirmed the whole transcriptomic data and provided information on the expression frequency of these genes. Tumor infiltrating Treg cells express with high frequency genes that are associated with increased suppressor activity, such as the well characterized OX40, CTLA4 and GITR. Moreover, there are a number of interesting and less expected genes the specific expression of which was validated also at the protein level. For example, IL-1 R2 upregulation could be another mechanism that tumor resident Treg cells employ to dampen anti-tumor immune responses through the neutralization of IL-1β function on effector cells. PD-L1 and PD-L2 expression has been recently reported on activated T cells or APCs (Boussiotis et al., 2014; Lesterhuis et al., 2011; Messal et al., 2011) but, to the best of our knowledge, neither PD-L2 nor PD-L1 expression has ever been reported in Treg cells, and our finding that they are overexpressed in tumor infiltrating Treg cells adds an additional level of complexity to the PD1/PD-Ls immunomodulatory axis within the tumor microenvironment. BATF is a transcription factor that has been mainly associated to Th17 development and CD8.sup.+ T cells differentiation (Murphy et al., 2013). Our findings show that BATF transcript is upregulated in tumor infiltrating Treg cells more than in tumor infiltrating Th17 cells (FIG. **8**). Interestingly, expression of BATF in CD8.sup.+ T cells is induced by IL-21 (Xin et al., 2015), and it was found that IL21R is highly expressed in tumor-infiltrating Treg cells (FIG. **4**).

[0259] It was showed that tumor infiltrating Treg cells express high amounts of 4-1 BB (CD137) a marker of TcR mediated activation (Schoenbrunn et al., 2012) and have shown they display very high suppressor function on effector T cell proliferation. It could be that expression of the signature genes correlated with the enhanced suppressive ability and so contributed to the establishment of a strong immunosuppressive environment at tumor sites. A corollary to our findings would have that increased number of Treg cells in the tumor environment should associate with a worst clinical outcome. In fact, when LAYN, MAGEH1 and CCR8 (which represent three of the most enriched genes in tumor infiltrating Treg cells) are highly detected in whole tumor samples there is a significant worsening of the 5 years survival of both CRC and NSCLC patients. Although, the functional roles in Treg cells of LAYN, a transmembrane protein with homology to c-type lectin (Borowsky and Hynes, 1998), and of MAGEH1, a member of the Melanoma Antigen Gene family (Weon and Potts, 2015) are unknown, the high expression of the chemokine receptor CCR8 is instead intriguing. Indeed CCL18, the ligand of CCR8 (Islam et al., 2013), is highly expressed in different tumors including NSCLC (Chen et al., 2011; Schutyser et al., 2005). The high specificity of CCR8 expression on tumor infiltrating Treg cells suggests it could be a new interesting therapeutic target to inhibit Treg cells trafficking to tumor sites, without disturbing recruitment of other effector T cells that do not express CCR8. Considerable efforts have been recently put in the development of sophisticated bioinformatics approaches that exploit lymphocyte gene expression data to understand the immune-modulatory networks at tumor sites, to predict clinical responses to immune-therapies, and to define novel therapeutic targets (Bindea et al., 2013a; Bindea et al., 2013b; Gentles et al., 2015). The data here presented represent the first comprehensive RNA-sequencing analysis performed on tumor-infiltrating human CD4.sup.+ Treg, Th1 and Th17 cells. Our findings highlight the relevance of assessing gene expression patterns of lymphocyte at tumor-sites and suggest that generation of more transcriptomic data of tumor-infiltrating lymphocyte subsets purified from different cancer types may contribute to a better understanding of the

dynamics underlying immune modulation in the tumor microenvironment. Moreover, our data represent a resource to generate and validate novel hypotheses that will increase our knowledge on tumor infiltrating Treg cell biology and should lead to the identification of new therapeutic targets.

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Claims

1.-**25**. (canceled)

26. A method for identifying an antibody acting as an anti-tumoral agent that depletes tumor-

infiltrating regulatory T cells, comprising the steps of: a) assaying candidate antibodies for their binding specificity to CCR8; b) selecting antibodies having a specific binding activity to CCR8; c) testing the specific binding antibodies in a cell system comprising tumor infiltrating regulatory T cells for their capacity of inhibiting proliferation and/or inducing an apoptotic response of the tumor infiltrating regulatory T cells.

27. The method of claim 26, wherein the depletion of tumor-infiltrating regulatory T cells comprises inducing antibody-dependent cell-mediated cytotoxicity (ADCC).

28. An in vitro method for monitoring the efficacy of a therapeutic treatment in a subject of a solid tumor which is a non-small cell lung cancer or a metastasis derived therefrom, said method comprising the steps of: a) obtaining an isolated biological sample containing tumor infiltrating T regulatory cells from the subject; b) detecting CCR8 in said biological sample; c) comparing the detected CCR8 to a control selected from a biological sample obtained from the same subject before initiation of the therapeutic treatment or taken at a time during the course of the therapeutic treatment, wherein a lower amount of CCR8 in the biological sample than in the control indicates effective treatment of the tumor.