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Domatinostat’s impact on tumor immunophenotype and response to PD-(L)1 and LAG-3 blockade in pre-clinical models
CONFLICT OF INTEREST STATEMENT

I hereby declare that

- I do not conduct activities that would involve a conflict of interest with CME-accordable training
- I’m employee of 4SC AG developing domatinostat for anti-cancer therapy
Epigenetic modulators in Cancer immunotherapy

Immunity continuum

non-inflamed

No immune response

Immunosuppression

T cell exclusion

T cell exhaustion

inflamed

TLR/RIG-I/STING
Oncolytic viruses
Tumor vaccines

CIT

CAR/ACT
CPI
4-1BB

Challenge

Immune response initiation

MDSC/M2 MF
T regs

T cell recruitment

PD-1/PD-L1
T cell memory

Epigenetic inhibitors

EZH2/DNMT/HDAC

DNMT/HDAC
EZH2/HDAC class I

EZH2/DNMT/HDAC

HDAC/EZH2/BET
BET

TUMOR ELIMINATION
Domatinostat

• Class I selective HDAC inhibitor

• Well tolerated as monotherapy
  – Tolerability in combination with pembrolizumab under evaluation (SENSITIZE, NCT03278665)

• Efficacy in monotherapy
  – 83% DCR
  – 1PR, 1CR
Responsivity to PD-1

- Tumor cell antigenicity and T cell inflammation ⇒ predictive of response to PD-(L)1 blockade
- Only a proportion of tumors respond even in highly antigenic tumors
  - Role of immunosuppression: MDSCs and M2 macrophages?

Cristescu et al., *Science*, 2018
Animal models for evaluation of domatinostat

- What is the immunomodulatory features of domatinostat in different tumor immune phenotypes?
  - Tumor models with $\text{TMB}^{\text{high}}$
  - Low response to PD-(L)1 blockade

- CT26 and C38 tumor models
  - Both high mutational load*
  - CT26 additionally express viral gp70

*Mosely et al., 2017, Cancer Immunology research
**CT26 model**

**BALB/C, blood, tumor-free animals**
- CD8
- CD4
- NK cells
- myeloid
- B cells

Total = 99.9

**BALB/C, blood, s.c. CT26 tumors**
- CD8+ T cells
- CD4+Foxp3- T cells
- Tregs
- myeloid cells
- other immune cells

majority Gr1^+Ly6G^+

Total = 96.75

**BALB/C, TME of s.c. CT26 tumors**
- CD8+ T cells
- CD4+Foxp3- T cells
- Tregs
- myeloid cells
- other immune cells

35% of tumor

**Blood**

- **CT26 (BALB/C background):**
  - increased number of GR1^+Ly6G^+ in blood
  - up to 43% immune cells in the tumor
  - Low number of CTLs in TME (~0.2%)
  - High number of CD206^+ MF in the TME
  - PD-L1 positive
  - No response to PD-(L)1 blockade (RMP1-14, 10F9G2, treatment start at 150-200 mm^3)
Domatinostat strongly increased the number of CTLs in the TME of CT26 tumors

- Strongly increased number of cytotoxic CD8\(^+\) T cells in the TME (not in blood)
- IHC showed that CTLs were inside the tumors

### Correlations: gene expression vs. CTLs and tumor volume

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<th>CTLs in tumor</th>
<th>Tumor volume</th>
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Domatinostat increases expression of MHC I and antigen-presenting machinery genes in CT26 model.
Domatinostat induced expression of PD-1 blockade response signature genes in CT26 model

Domatinostat + anti-PD-L1 combination resulted in reduced tumor growth and increased survival in CT26.

**Tumor growth**

- Vehicle
- Anti-PD-L1
- Domatinostat
- Combination

**Survival**

- Vehicle
- PD-L1
- Domatinostat
- Combination

* ****
** *
* n.s.*
C38 (C57BL/6 background):
- Daughter cell line of MC38 passaged in mice
- Tumor inoculation using tumor fragments
- Low number of immune cells in the TME, majority CTLs
- Barely any immunosuppressive immune cells in the TME
- BUT the response to PD-1 blockade limited (10-25%)
Domatinostat synergized with PD-1 blockade in C38 model

- vehicle
- anti-PD-1
- domatinostat
- domatinostat+anti-PD1

**tumor volume (mm³)**

- **days**
- **vehicle**
- **domatinostat**
- **domatinostat+anti-PD1**

**treatment start**
Domatinostat + anti-PD-1 blockade combination resulted in durable CR in C38 model.
In combination with anti-PD-1 blockade, domatinostat beneficially affect the quality of CTLs.
Domatinostat increased MHC class II expression on tumor cells as well as on M1 macrophages.
Rationale for the triple combination domatinostat+anti-PD-1+ anti-LAG3

• MHC-II positivity on tumor cells is associated with therapeutic response to PD-1 blockade, progression-free and overall survival, as well as CD4\(^+\) and CD8\(^+\) tumor infiltrates (Johnson et al., 2016, *Nat Commun*)

• Domatinostat induces expression of MHC class II and co-stimulatory molecules on tumor cells and macrophages potentially enhancing tumor-specific response

• MHC class II is the ligand for the T cell inhibitory receptor LAG3

➢ A triple combination of domatinostat with anti-PD1 and anti-LAG3 antibody should beneficially affects T cell response by supporting T cell activation and function
Triple combination of domatinostat + anti-PD-1 + anti-LAG3 resulted in superior anti-tumoral effect
Triple combination of domatinostat + anti-PD-1 + anti-LAG3 resulted in more CR

More regressions compared to domatinostat and anti-PD-1 monotherapies

Nearly all tumors regress
Summary

- HDAC class I specific inhibitor domatinostat:
  - increased MHC class I and components of APM in vivo
  - In CT26 (low basal number of CTLs)
    o Increased number of CTLs
    o increased predictive gene signatures for response to PD-1 blockade
    o In combination with PD-L1 blockade significantly increased survival time
  - In C38 (high basal number of CTLs):
    o increased proliferative capacity of CTLs
    o reduced ratio of exhausted/total CTLs
    o Increased MHC class II on tumor cells and M1 MF
    o In combination with PD-1 blockade induced survival rate with ~ 60% durable CRs
    o Triple combination with PD-1/LAG3 blockade further increased survival rate with ~ 80% durable CRs
Many thanks

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4SC Research group
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