Cytokine release data
- regulatory experience in the EU

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The presented views are my personal views and do not necessarily represent the view of the Paul-Ehrlich-Institut or any other regulatory body.
Approach

• Review of FIH clinical trial applications for mAbs (mAbs, Fab, scFv, bi-specific molecules)

• For which type of mAbs was cytokine release testing performed?

• Cytokine release assay formats? in vitro / in vivo

• What are the results? in vitro / in vivo results correlation with clinical data
mAbs according to targets / mode of action

- soluble / blocking
- cellular R / blocking
- cellular R / activating or agonist
- cellular R / cytotoxic

cytokine release
Soluble target – blocking

- number of programmes: 13
- cytokine assessment in vitro: 10x no, 3x yes
- result of cytokine assessments: 3x negative
- result in clinical trial: where data are available, no evidence for cytokine release

Based on MOA, cytokine release in vivo not expected confirmed by lack of cytokine release in clinical trials (consistent with lack of cytokine induction in vitro)
Cellular target – blocking

- number of programmes: 7
  (6 products modified to reduce/eliminate Fc effector function)

- cytokine assessment in vitro: 7x yes

- result of cytokine assessments:
  5x negative, 2x positive (minimal levels)

- cytokine evaluation in NHP tox studies: 6x no, 1x yes
  no elevation of cytokines

- cytokine monitoring in clinical trials: listed in protocol for 2 products

- result in clinical trials: 5 studies
  4x no evidence for cytokine release / infusion reaction
  1x infusion-related reaction associated with IL-6, IL-8 production
  (negative in vitro results)

Negative in vitro result not necessarily predictive for situation in humans
Cellular target - cytotoxic mAbs

- number of programmes: 5
  (all Fc-modified to enhance effector function)

- cytokine assessment in vitro: 5x yes
  human whole blood, soluble Ab, in general large panel of cytokines
  IL-1, 6, 8, TNFa, 10, 12, IFNg (IP10), GM-CSF, IL-2

- result of cytokine assessments: 5x positive
  in all studies slight elevations of cytokines IL-6, IL-8, TNF-a
  effect less strong than for alemtuzumab (if included)

- cytokine evaluation in NHP tox studies: 2x no, 3x yes
  1x positive elevated levels of IL-6 in RD tox

- impact on clinical protocol:
  cytokines measured in all trials

- result in clinical trials (4x anti-cancer, 1x auto-immune)
  IRR seen in all trials (1st dose effect)

Cytokine release in vitro consistent with infusion-related reactions in humans
mAb with agonistic / immunostimulatory activity

- number of programmes: 4
  bi-specific molecules (T vs. tumour target)

- cytokine assessment in vitro: 4x yes
  assay set-up dependent on the specific product
  PBMC + tumour target,
  in general focus on effector cell activation
  IFNγ, IL-2, TNF-α, IL-6, IL-10

- result of cytokine assessments: 4x positive
  in the presence of tumour cells, activation of effector cells and
  concomittant cytokine release

- cytokine evaluation in NHP tox studies (where feasible)
  transient cytokine release, flu-like symptoms

- impact on clinical protocol:
  cytokines measured in all trials, pre-medication, dosing schedule

Cytokine release is expected based on effector mechanism
T cell-specific mAb with reduced Fc activity

- number of programs: 3
  anti-CD3, Fc-modified to prevent interaction with Fcγ receptors
- In vitro: non-mitogenic, induction of cytokines absent / strongly reduced
- Cytokine release syndrome in humans
- Absence of cytokine release in vitro does not indicate absence of cytokine release syndrome in vivo
  predictive value of negative in vitro result ??
Recommendations

• For mAbs that block soluble targets (lack of evidence for cytokine release in vivo) cytokine assessment in vitro generally not warranted

• Cave!
  If membrane form of the target or mAb-Ag complex can bind to cellular R, in vitro cytokine testing warranted
Recommendations

• For mAbs targeting cellular receptors, in vitro cytokine assessment is warranted

• In addition, take into account MOA to evaluate the cytokine inducing risk of a product
  - blocking
  - cytotoxic
  - immunostimulatory

• However, for cytotoxic / immunostimulatory mAbs in case of negative cytokine data
  MOA prevails with regard to risk assessment
Cytokine release assays

(adapted from Stebbings et al., 2007)
Cytokine release assay – recommendations

• Cell population: whole blood in general healthy donors; consider patients (e.g. autoimmune diseases)

• mAb presentation:
  - soluble and plate-bound (wet)
  - other forms of cross-linking also acceptable (on beads)

  - dry-coating is usually not requested
  - use of co-culture with endothelial cells not requested

• Different assay set-up for products targeting CD3 or costimulatory receptors/ligands on T cells

  based on pharmacology of the product
Cytokine release assay – recommendations

- Read-out: no recommendations made
  ELISA, MSD multiplex, BD cytokine bead array, ...

- Cytokines to be measured:
  IL-6, IL-8, TNF-a
  in addition (dependent on target/MOA) consider:
  IL-1, IFNg (IP-10), IL-2, IL-12, IL-10, Th2 cytokines

- Comparators:
  dependent on target / MOA;
  pos. control: OKT-3, alemtuzumab, rituximab
  ideally also neg. control (e.g. palivizumab)
  LPS, PHA
Open question

• Possible to define "threshold" level of cytokines associated with human risk?

• Relative expression in relation to comparator