

Blood sample collection in the SCCS cohort

Regular Visits

1. Plasma (3 aliquots per visit)

One 6 ml EDTA tube for 3 aliquots of plasma (of 0.9 ml each, e.g. TPP cryotubes No. 89012)

2. Cell pellets for host DNA storage (3 aliquots per visit, for at least 3 visits, then stop). Centres may use one of the two procedures described below, at their own choice:

- **CPT tubes**

Two 4 ml CPT Tubes (Cell Preparation Tube, Becton Dickinson, No. 362760)

- Tubes are sent to the laboratory without centrifugation
- Centrifugation for 20 min at 2300 g (e.g. Sorvall H 4000 swingout, 3000 rpm)
- Supernatant of both tubes is transferred & pooled in a 15 ml conical polypropylene tube (e.g. Falcon "blue cap")
- Centrifugation for 20 min at 2300 g
- Separate the supernatant from the cell pellet
- Use a small aliquot (approx. 300 µl) of supernatant to resuspend the cell pellet in the 15 ml tube
- Make at least three aliquots, transfer to cryotube (e.g. Nunc No. 363401), freeze and store at -80° C

- **EDTA tubes**

Two 6 ml EDTA tubes

- Centrifuge whole blood at 2000 rpm, 10 min, RT and recover the plasma
- Bring the remaining volume to 30 ml with PBS + 2 mM EDTA
- Transfer carefully the 30 ml on top of 10 ml Ficoll-Paque™ PLUS (1.077 g/ml, Amersham Biosciences) by using a pipette
- Centrifuge at 2000 rpm, 20 min, RT
- Harvest PBMCs from the interface by using a pipette
- Wash the cells with 40 ml of PBS + 2 mM EDTA
- Centrifuge at 1600 rpm, 10 min, RT
- Repeat steps 6 and 7
- Resuspend cells in 1 ml FBS + 10% DMSO
- Make at least three aliquots and store at -80 °C until use

Treatment related visits (baseline, week 2, week 4, week 12, week 24 [if applicable], end of treatment [if not 12 or 24 weeks], 12 weeks after the end of treatment)

1. Plasma (optional)

One 6 ml EDTA tube for 3 aliquots of plasma (of 0.9 ml each, e.g. TPP cryotubes No. 89012)