



CMS - forskning på sykdomsmekanismer og markører

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NFR-BIP prosjekt 187301/S40: 2008-2011

'Cardiomyopathy syndrome: A multi-task approach to reduce losses & improve knowledge'

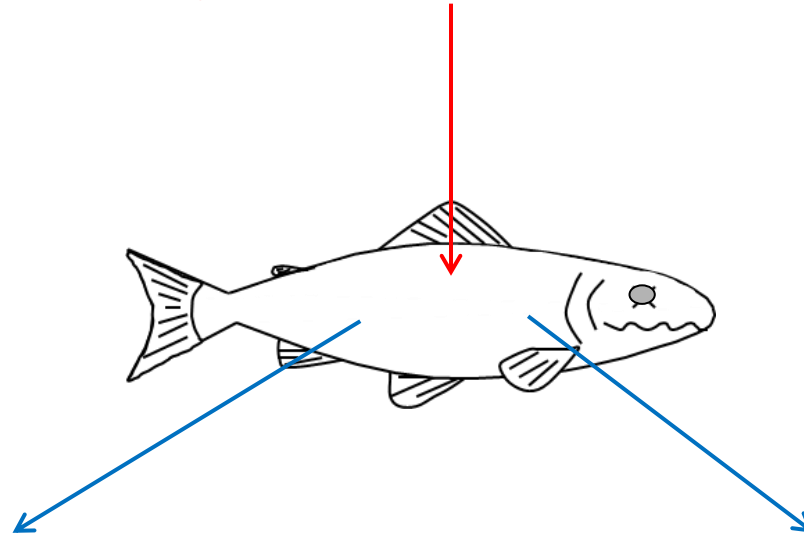
Finansiering: NFR, industripartnere & FHF



Bakgrunn CMS - FoU utfordringer

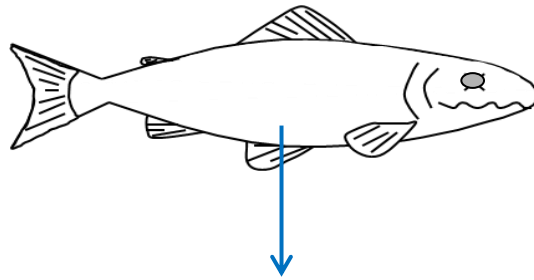
- Ukjent etiologi
 - Virus
 - Metabolsk syndrom
 - Autoimmunitet
 - Første smitteforsøk: overførbar sykdom (Fritsvold et al. 2009)
- Differensiell diagnostikk
 - Overlappende patologi med HSMB and PD
- Sykdomsovervåkning
 - Forbedre diagnostiske verktøy (tidlig fase)
 - Risiko faktorer (miljø, geno/fenotype)
- Nå: kausalt virus identifisert (PMCV) (Haugland et al., 2011)

Ukjent sykdomsfaktor



Fenotypiske responser:
-Kliniske tegn
-Patologi, vevsskade

Molekylære responser:
-Gener reguleres (av/på)
-Proteiner/enzymer reguleres

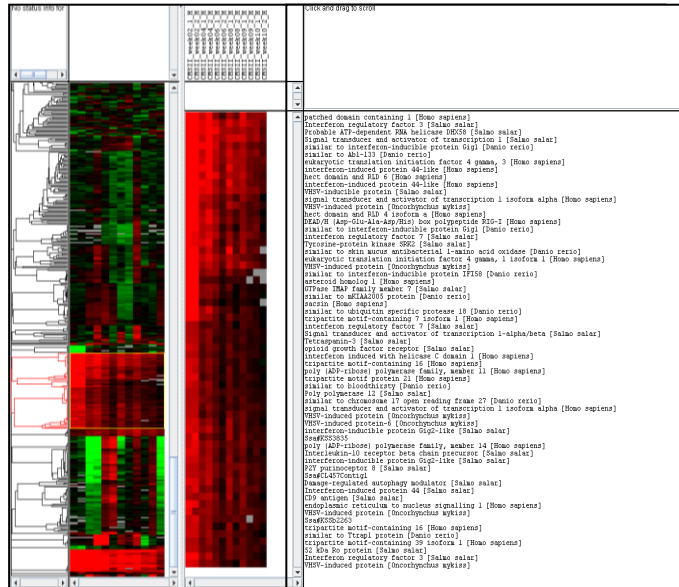


Molekylære responser - Microarray/RT-qPCR:

- Hvilke gener er regulert?
- Hvordan er de regulert?

Funksjon:
 -Immunforsvaret
 -Komplement
 respons
 -IFN reseptor

MEKANISMER



'Egenskap':
 -Virus-infeksjon
 -HSMB
 -Temperatur
 -Robusthet
 -Etc..

MARKØRER

Smitteforsøk:



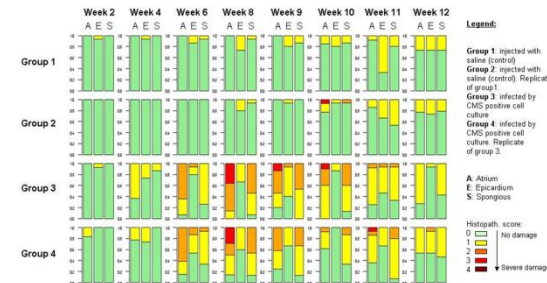
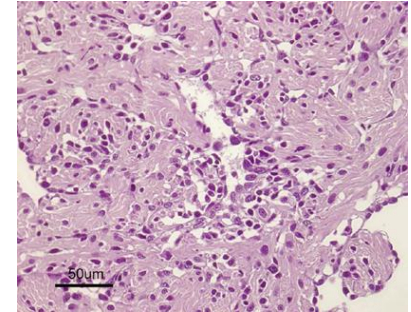
Pilotforsøk (NIVA Solbergstrand, 2008):

- Villaks Numedalslågen, 50-100 gr
- Ulike smittestoff , injeksjon/kohabitasjon
- Tidsuttak, organer etc



Hoved-smitteforsøk (VESO Vikan, 2009):

- Std uvaksinert post-smolt, 50 gr
- Celle-inokulat, ip-injeksjon
- Uttak alle vev, 8 tidspunkt



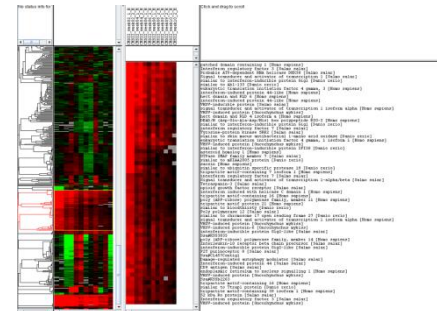
Feltmateriale:



Kasus-kontroll forsøk (2008-11):

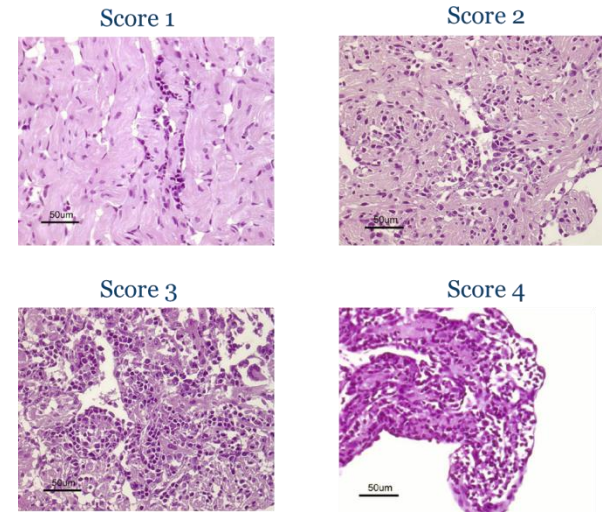
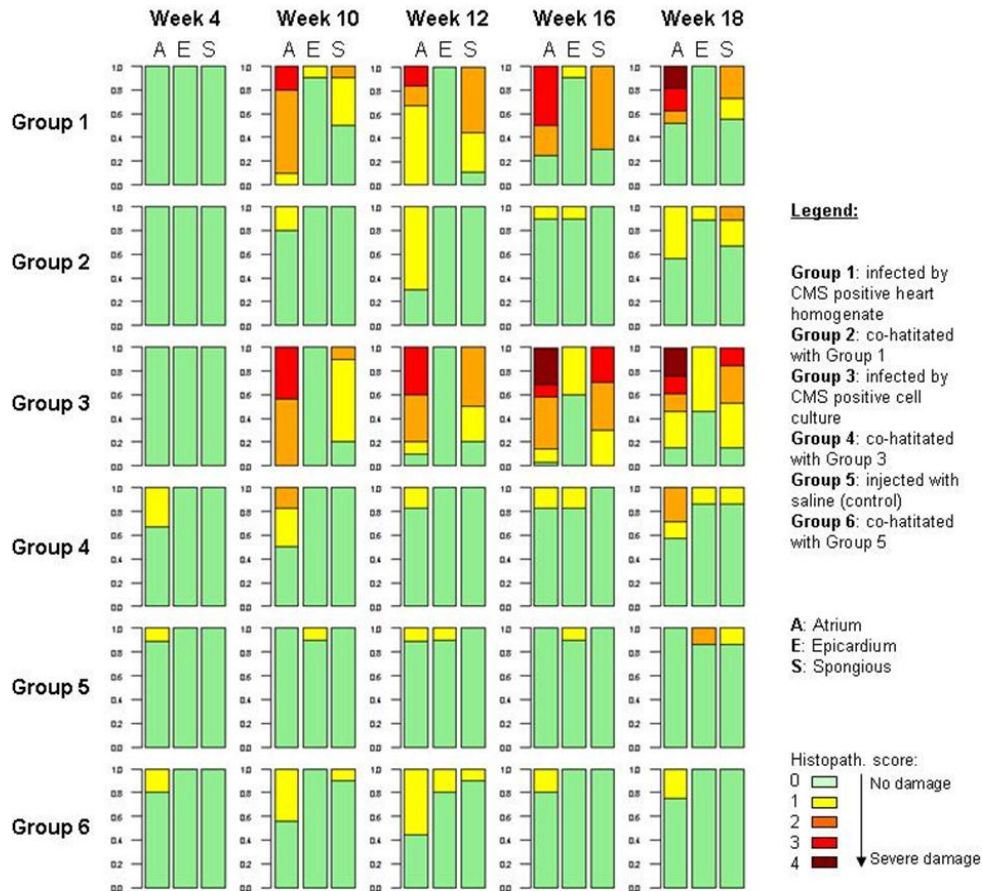
- Prøver fra CMS utbrudd og ktrl anlegg
- Felt-korrelat for resultater fra smitteforsøk

Fisk fra Aqua Gens avlsskjerne

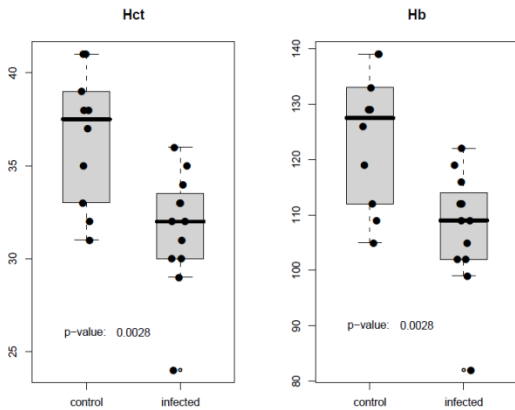


Pilot smitteforsøk

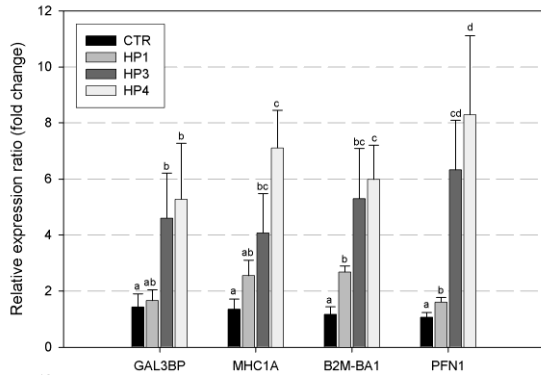
Histopatologi- NVI/NVH:



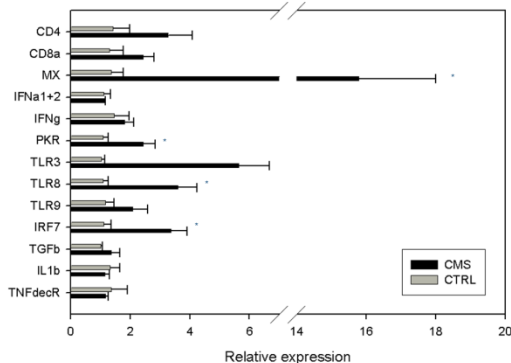
Photos: M Alarcon/TT Poppe



➤ CMS fisk utvikler anemi (reduisert Hct/Hb)



➤ Gener korrelert med histopatologi score gir grunnlag for diagnostiske markører



➤ CMS aktiverer gener som spesifikt responderer på virus-infeksjon- indikasjon på etiologi

Hoved-smitteforsøk

Fish: Unvaccinated Atlantic salmon smolt, aver size 50 gr (VESO Vikan)

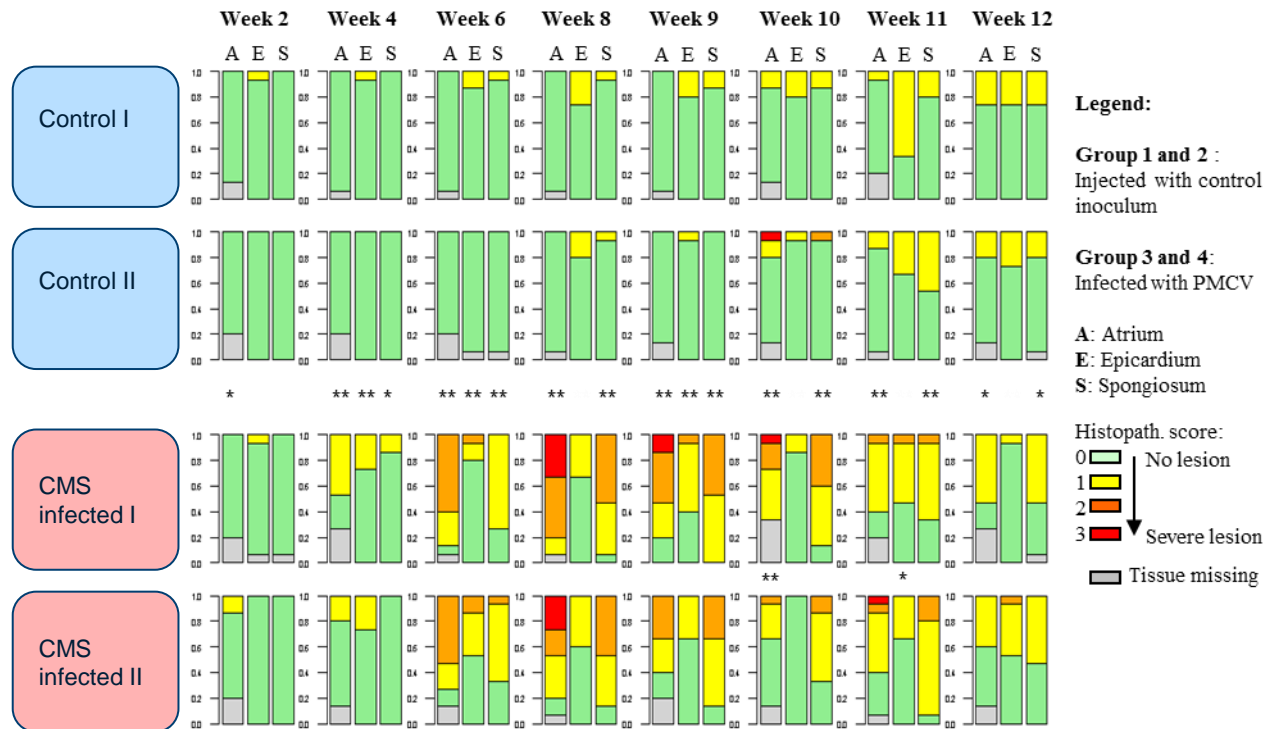
Control groups:
Injected with control inoculate



Test groups:
Injected with CMS
inoculate (infected cell
culture)



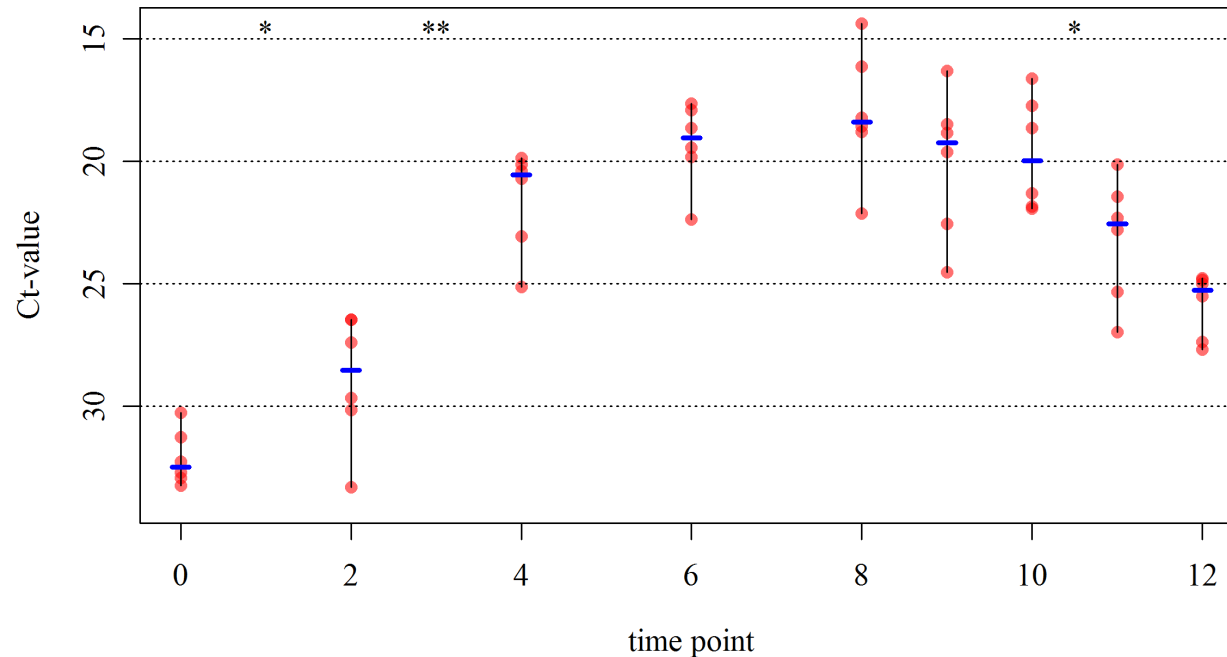
Histopatologi – hjertevev*:



- Ingen dødelighet
- Patologi uke 6-11, maks uke 8-9

*Utført av VI/NVH

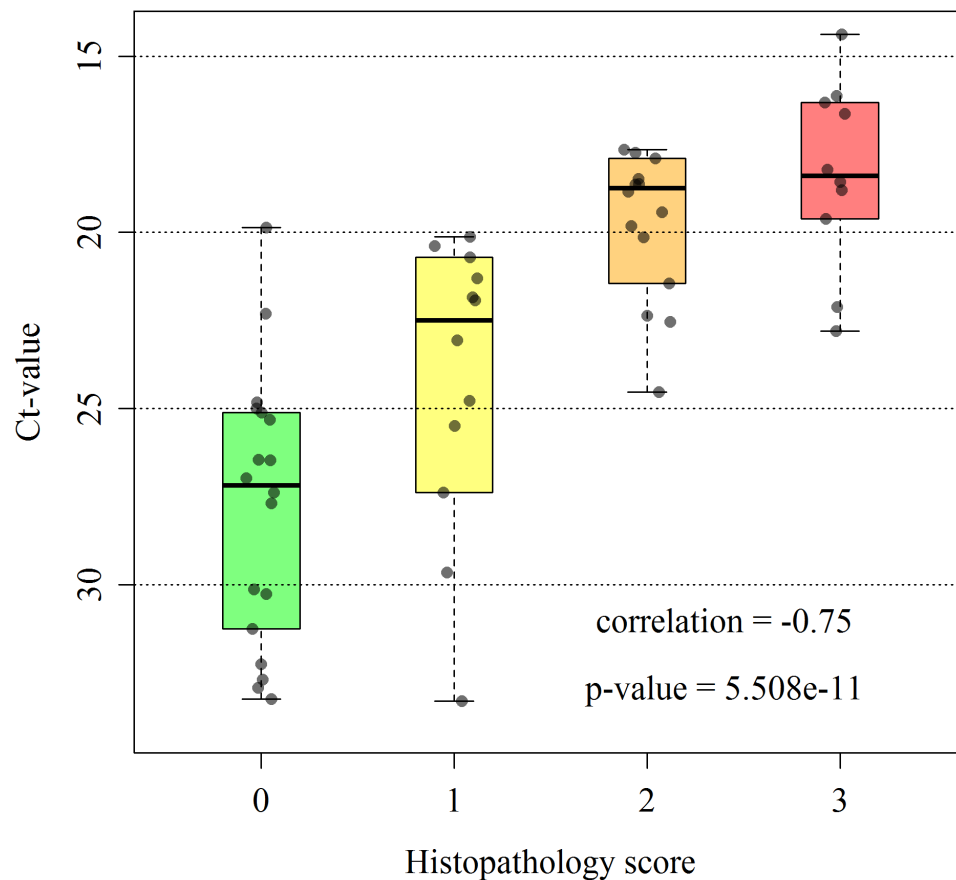
Mengde PMCV virus (RNA) i hjerte over tid (qPCR)*



*utført av
PHARMAQ

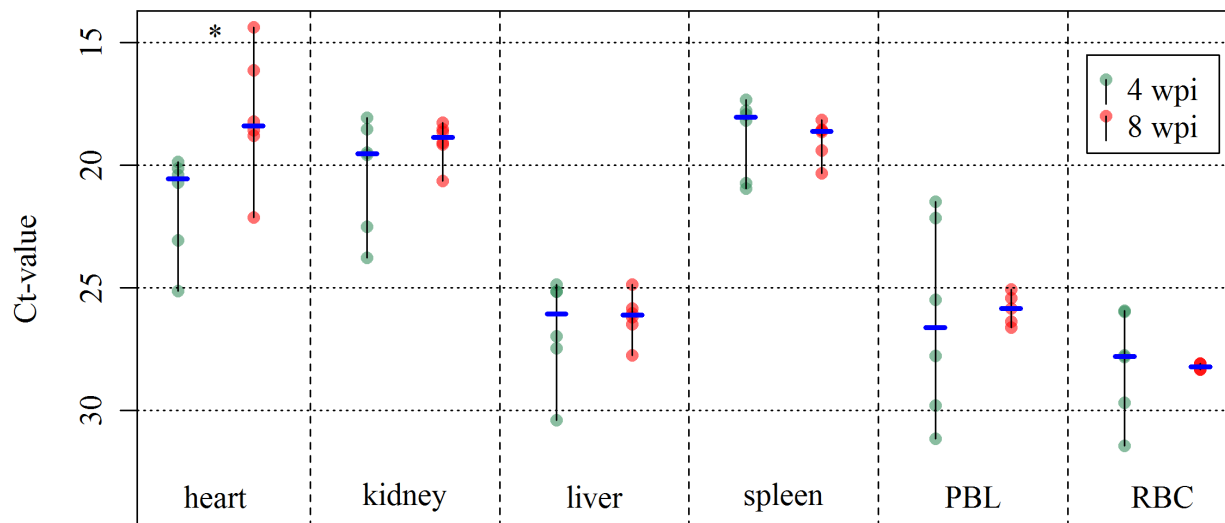
- Viruset formerer seg kraftigst i tidlig fase (fra uke 2-4)
- Platå-fase sammenfaller med histopatologi-topp
- Verten kvitter seg med virus fra uke 10-12

Korrelasjon hjerte histopatologi og virus-mengde



- Grad av hjerteskkade bestemmes av mengde virus (cytopatiske effekter)

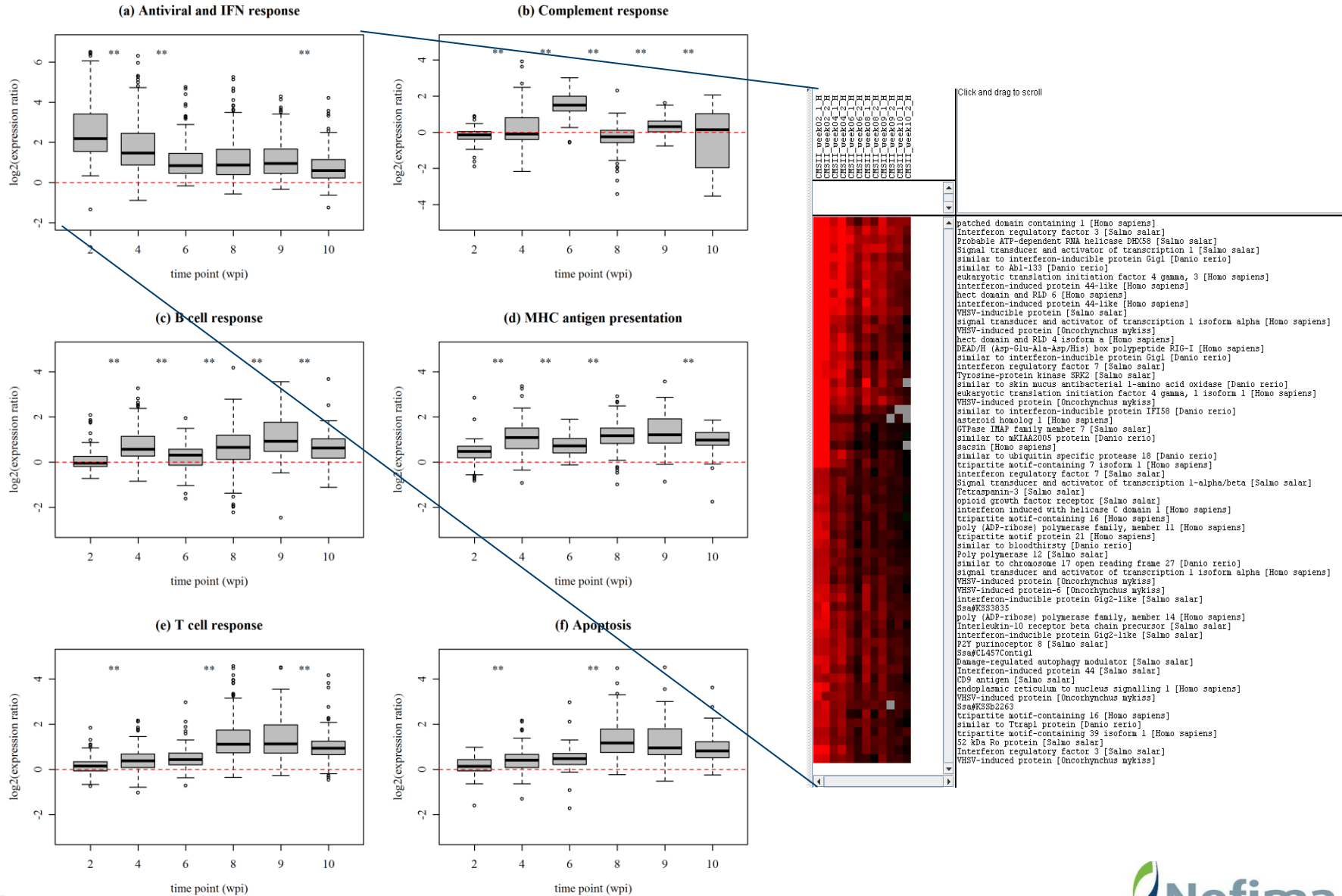
Mengde virus i ulike organer, tidlig og sen fase*



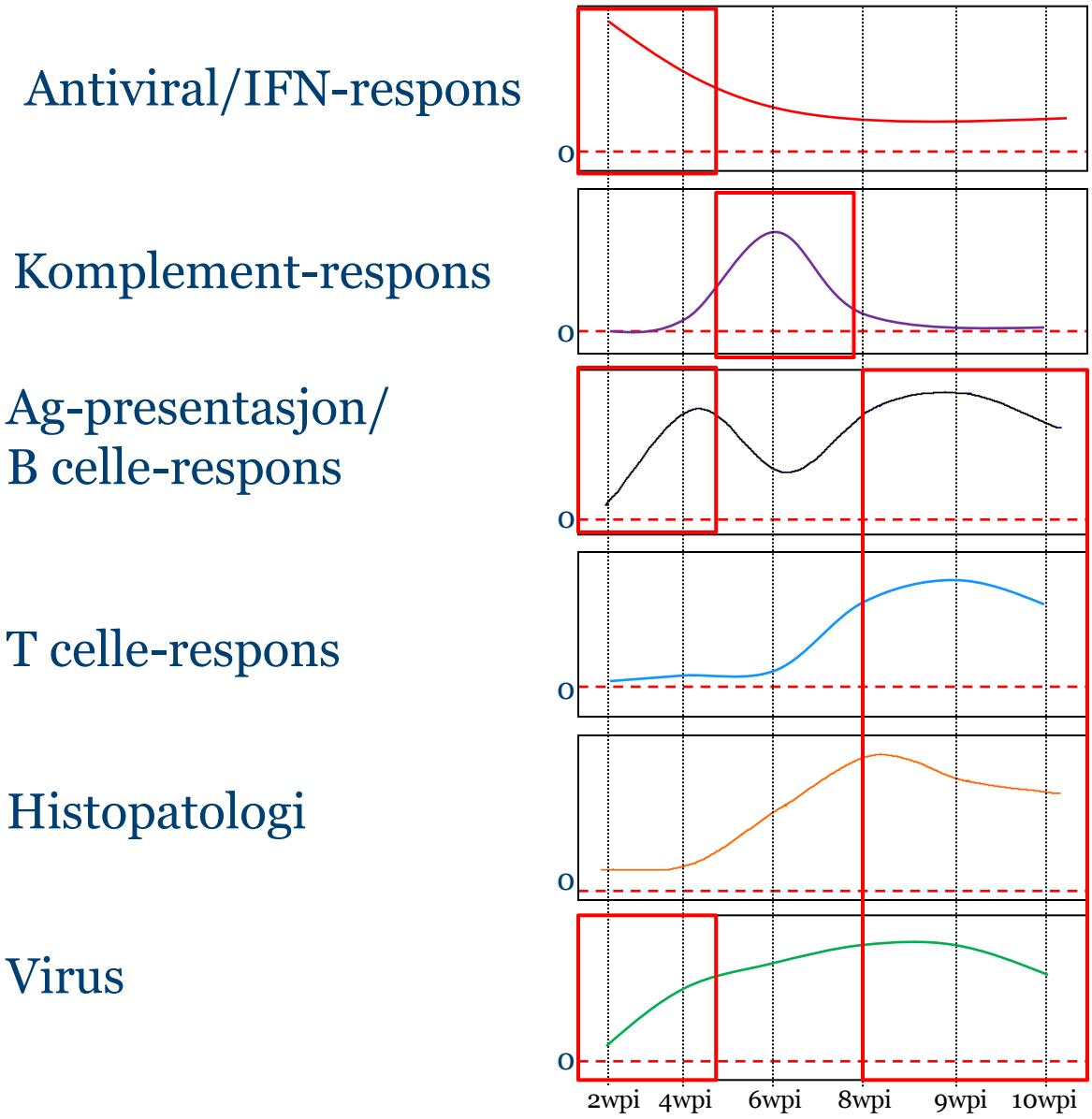
*utført av
PHARMAQ

- Viruset finnes i alle organer
- Dominerer i hjerte, nyre og milt
- Patologi i andre vev enn hjerte?
- Tidlig påvisning i blod – utg pkt for diagnostikk/overvåkning

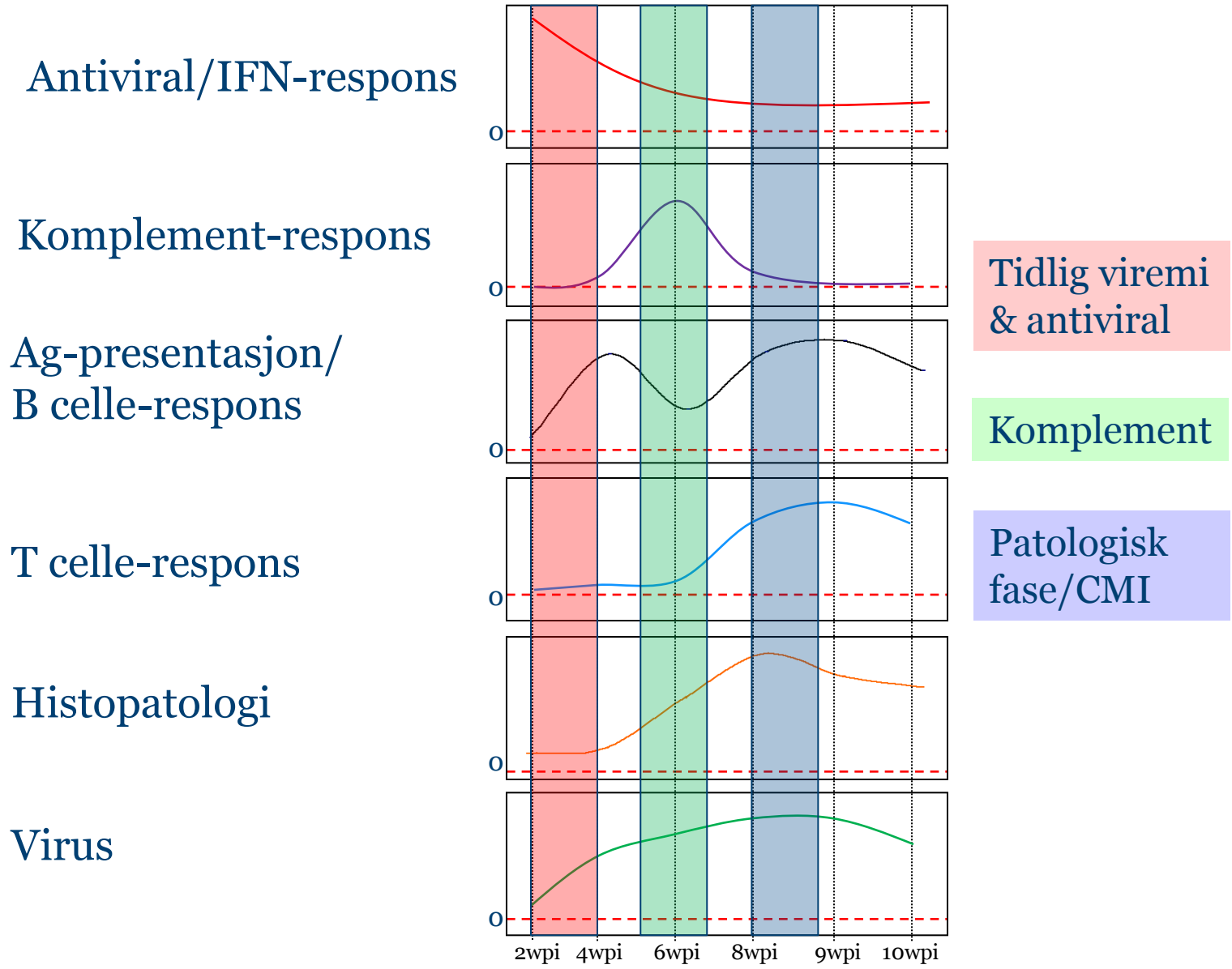
Immunologiske responser i hjerte (microarray)



CMS sykdoms-profil: Mekanismer

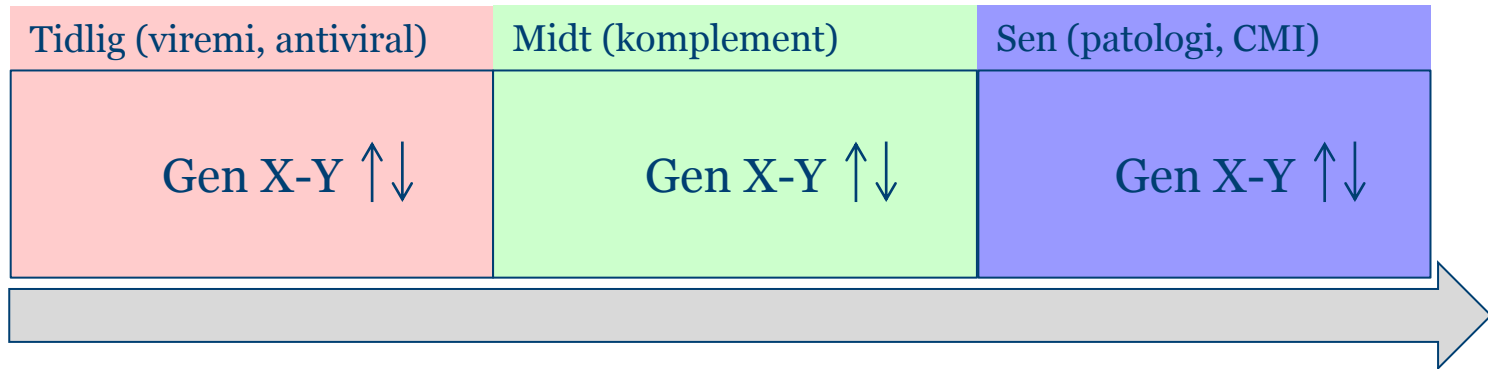


CMS sykdoms-profil: Markører

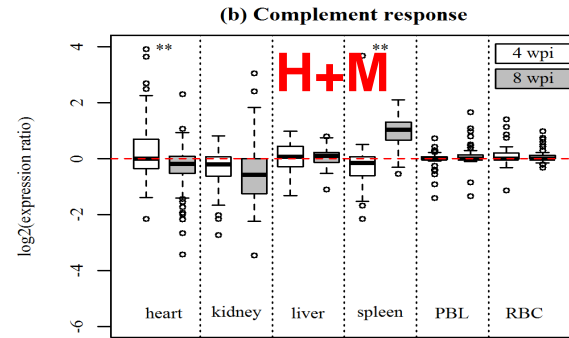
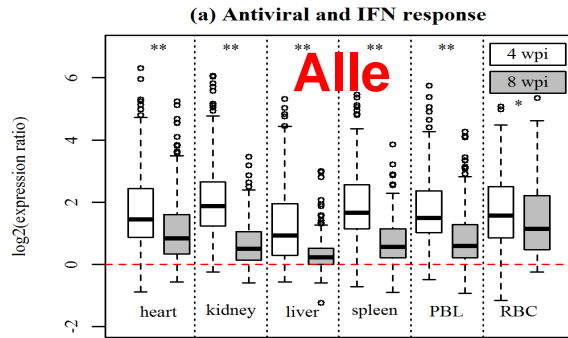


Etablert basis profil for sykdomsstadier

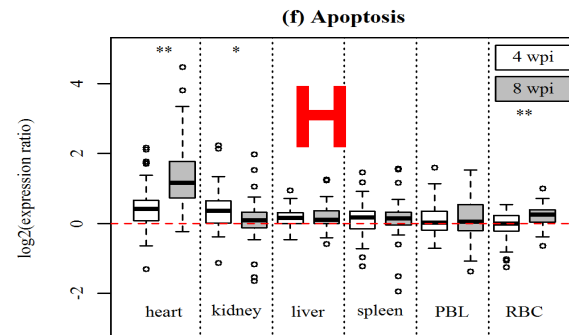
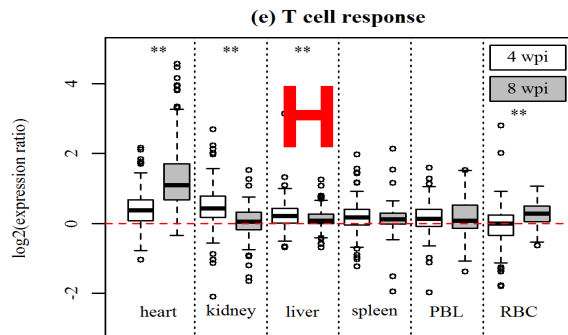
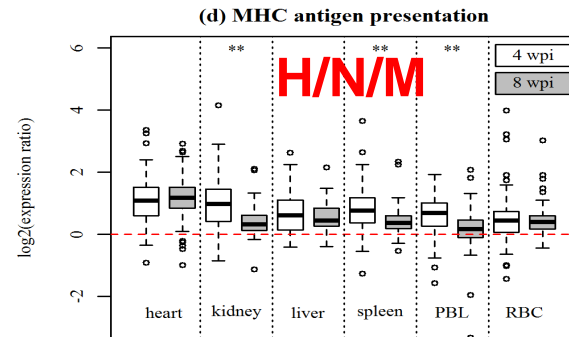
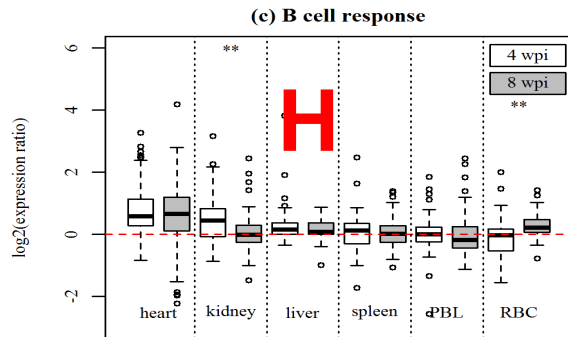
Gen-profiler = sykdomsmarkører



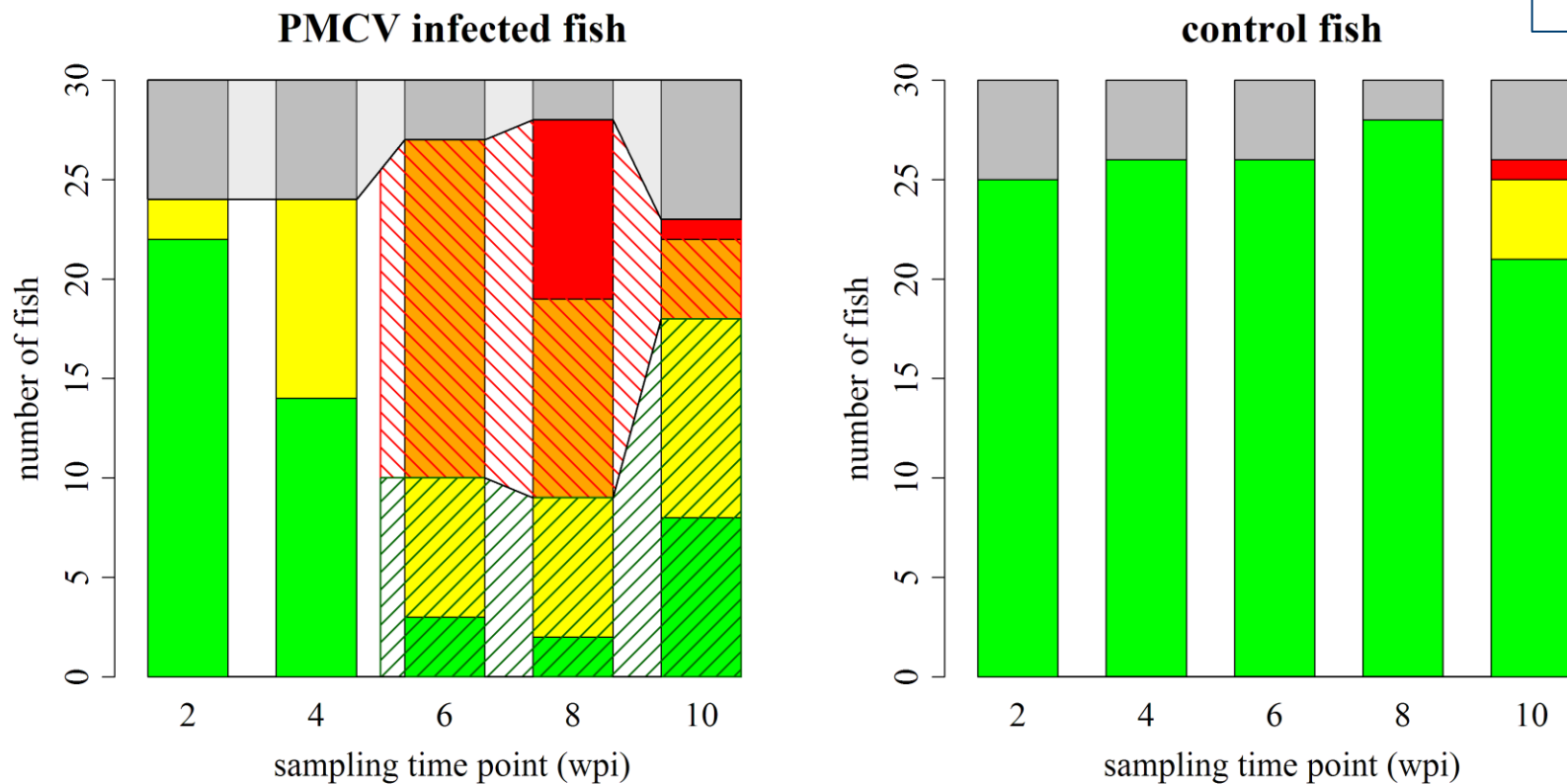
Immunologiske responser i ulike organer, uke 4+8



H - hjerte
N - nyre
M - milt

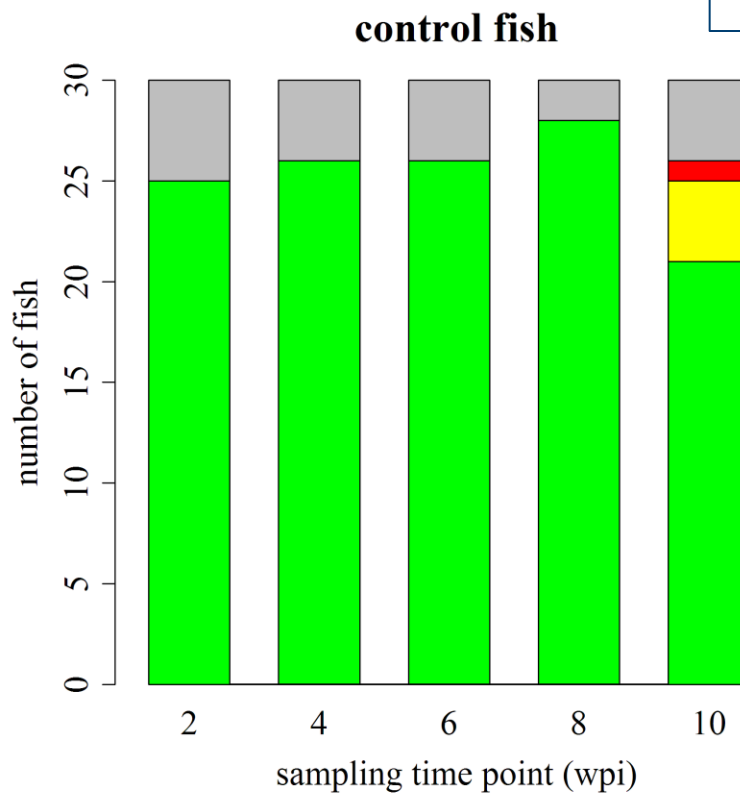
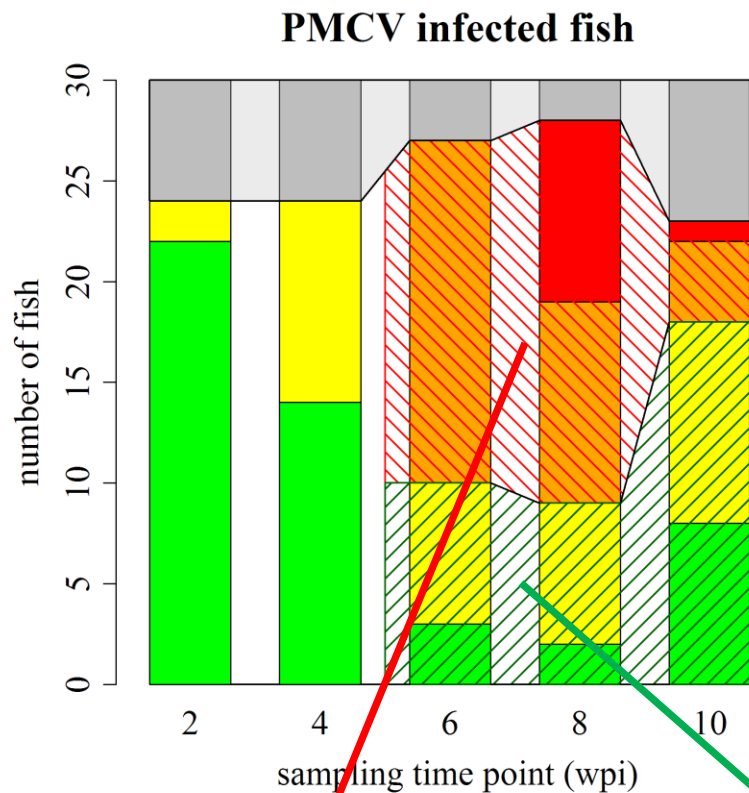
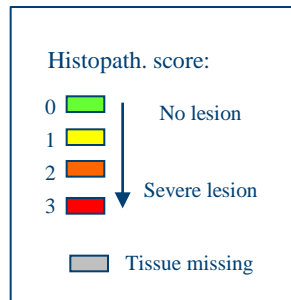


Fisk responderer ulikt på infeksjon:



➤ *Betydelig andel fisk utvikler ikke hjerteskkade*

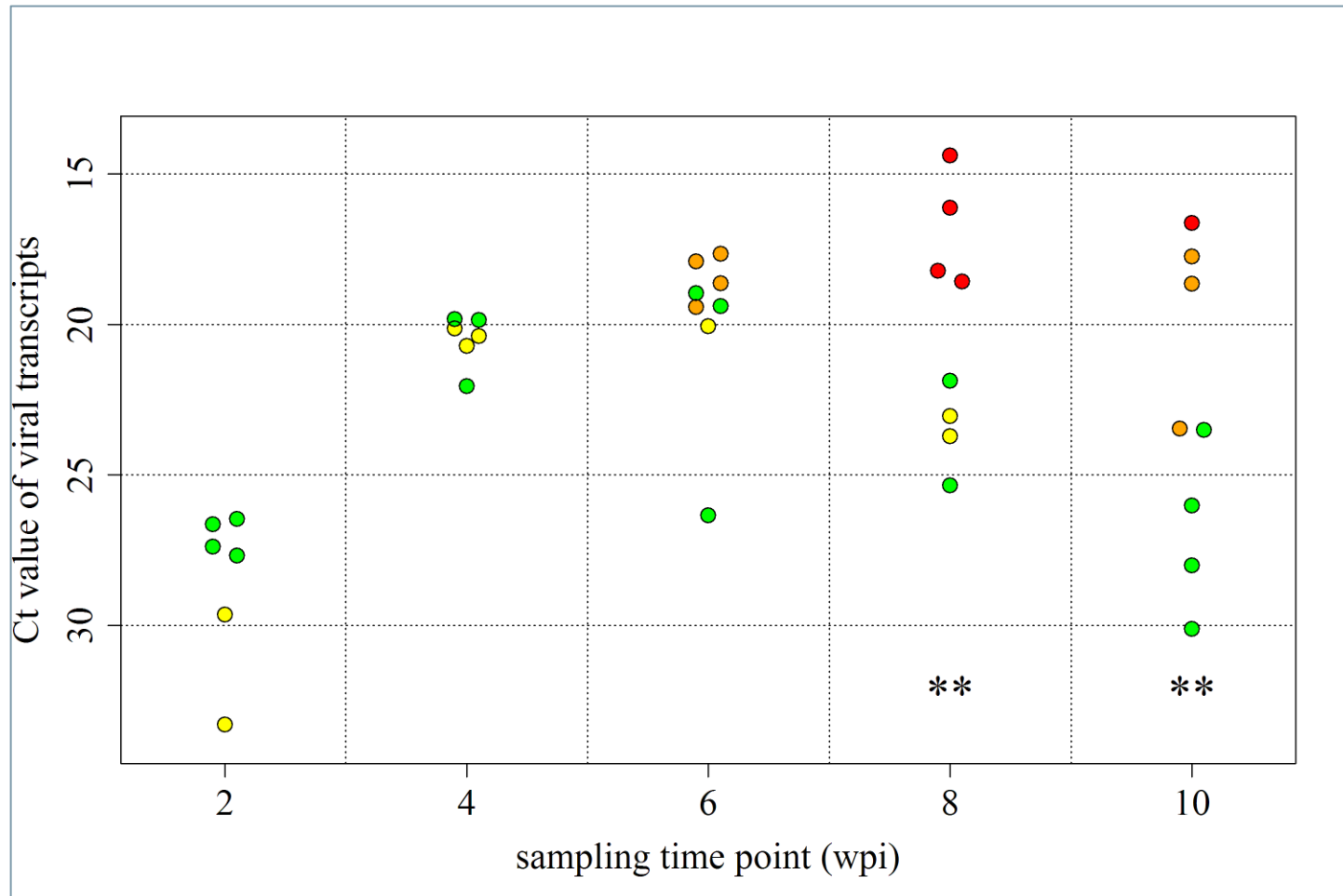
Resistens (immunologi) eller adferdsstrategi?

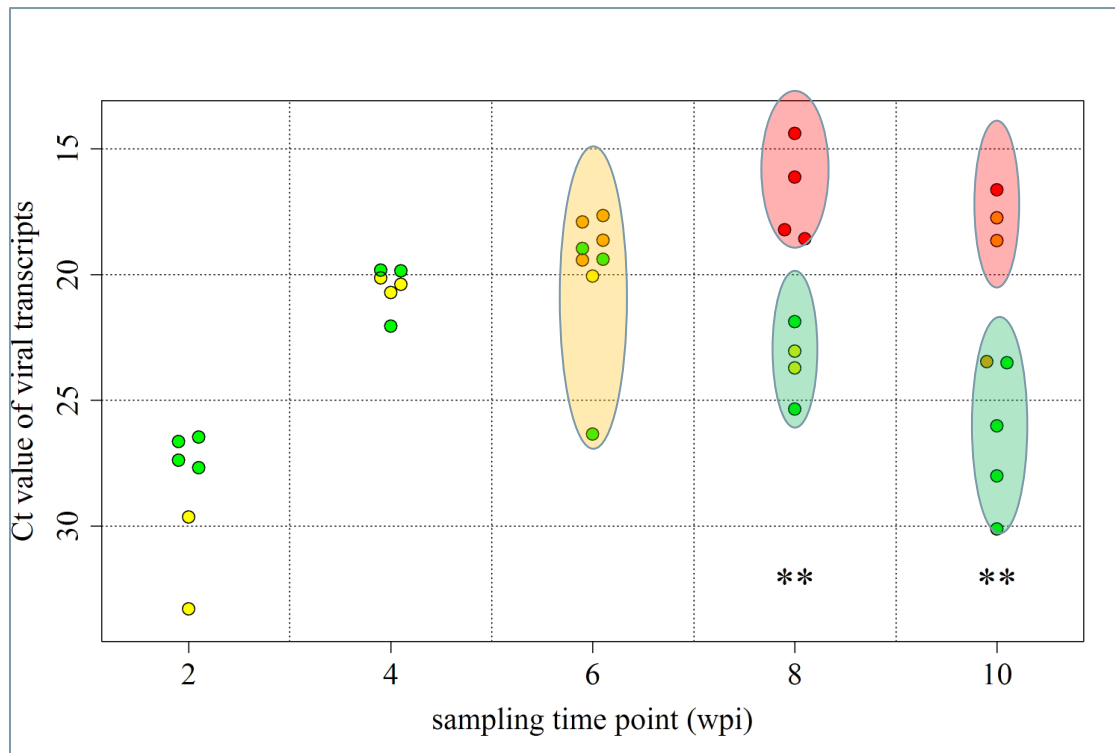


High Responders (HR)

Low Responders (LR)

Ulik mengde virus i HR vs LR?





- Like nivåer inntil uke 6
- Redusert virus-infeksjon assosiert med mangel på hjerteskaade (evnt recovery)
- Underliggende mekanismer?

Verts-responser i HR vs LR:

HR

LR

	2 wpi	4 wpi	6 wpi	8 wpi	10 wpi	
HR	<p>13 GO: 2376: immune system process 8 GO: 9607: response to biotic stimulus 5 GO: 19882: antigen processing and presentation</p>	<p>34 GO: 2376: immune system process 20 GO: 6955: immune response 12 GO: 19882: antigen processing and presentation 6 GO: 18212: peptidyl-tyrosine modification</p>	<p>17 GO: 2376: immune system process 11 GO: 6955: immune response 9 GO: 19882: antigen processing and presentation 3 GO: 2474: antigen processing and presentation of peptide antigen via MHC class I 3 GO: 48002: antigen processing and presentation of peptide antigen</p> <p>57 GOs significant: 5 GO: 14866: skeletal myofibril assembly 6 GO: 55002: striated muscle cell development 3 GO: 48739: cardiac muscle fiber development 3 GO: 30241: skeletal muscle myosin thick filament assembly 3 GO: 30240: skeletal muscle thin filament assembly 52 more ...</p>	<p>21 GO: 50896: response to stimulus 18 GO: 2376: immune system process 12 GO: 19882: antigen processing and presentation 12 GO: 6955: immune response 4 GO: 2474: antigen processing and presentation of peptide antigen via MHC class I 4 GO: 48002: antigen processing and presentation of peptide antigen 3 GO: 2483: antigen processing and presentation of endogenous peptide antigen 3 GO: 19885: antigen processing and presentation of endogenous peptide antigen via MHC class I 3 GO: 19883: antigen processing and presentation of endogenous antigen</p>	<p>5 GO: 6955: immune response 3 GO: 19882: antigen processing and presentation</p>	Histopathology 2 or 3
LR	<p>6 GO: 55001: muscle cell development</p>	<p>7 GO: 51056: regulation of small GTPase mediated signal transduction 6 GO: 35023: regulation of Rho protein signal transduction 5 GO: 14866: skeletal myofibril assembly 5 GO: 30239: myofibril assembly 5 GO: 31032: actomyosin structure organization 5 GO: 10927: cellular component assembly involved in morphogenesis</p>	<p>15 GO: 2376: immune system process 11 GO: 6955: immune response 8 GO: 19882: antigen processing and presentation</p> <p>61 GOs significant: 5 GO: 14866: skeletal myofibril assembly 3 GO: 48739: cardiac muscle fiber development 3 GO: 30241: skeletal muscle myosin thick filament assembly 3 GO: 30240: skeletal muscle thin filament assembly 3 GO: 71688: striated muscle myosin thick filament assembly 5 GO: 30239: myofibril assembly 3 GO: 7076: mitotic chromosome condensation 5 GO: 31032: actomyosin structure organization 53 more...</p>		<p>6 GO: 2376: immune system process 5 GO: 19882: antigen processing and presentation 5 GO: 6955: immune response</p>	Histopathology 0 or 1

*GO enrichment (funksjonelle gen-klasser)

Verts-responser i HR vs LR:

Immun-responser OPP
Hjertemuskelatur NED

Immun-responser OPP
T celle-respons

HR

LR

	2 wpi	4 wpi	6 wpi	8 wpi	10 wpi	
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LR	13 GO: 2376: immune system process 8 GO: 9607: response to biotic stimulus 5 GO: 19882: antigen processing and presentation 6 GO: 55001: muscle cell development	34 GO: 2376: immune system process 20 GO: 6955: immune response 12 GO: 19882: antigen processing and presentation 6 GO: 18212: peptidyl-tyrosine modification 7 GO: 51056: regulation of small GTPase mediated signal transduction 6 GO: 35023: regulation of Rho protein signal transduction 5 GO: 14866: skeletal myofibril assembly 5 GO: 30239: myofibril assembly 5 GO: 31032: actomyosin structure organization 5 GO: 10927: cellular component assembly involved in morphogenesis	15 GO: 2376: immune system process 11 GO: 6955: immune response 8 GO: 19882: antigen processing and presentation 61 GOs significant: 5 GO: 14866: skeletal myofibril assembly 3 GO: 48739: cardiac muscle fiber development 3 GO: 30241: skeletal muscle myosin thick filament assembly 3 GO: 30240: skeletal muscle thin filament assembly 3 GO: 71688: striated muscle myosin thick filament assembly 5 GO: 30239: myofibril assembly 3 GO: 7076: mitotic chromosome condensation 5 GO: 31032: actomyosin structure organization 53 more ...		6 GO: 2376: immune system process 5 GO: 19882: antigen processing and presentation 5 GO: 6955: immune response	Histopathology 0 or 1

Celle-mediert
immun respons:
-Reduserer virus
-Forsterker patologi

Immun-responser OPP
Hjertemuskelatur NED

Ingen immun-respons

Betydning for industrien?

- Basis profil - sykdomsmarkører
- Evaluere effekter av sykdomsbegrensende tiltak
 - Vaksinerings
 - Genetikk
 - Ernæring
 - Miljø
- Overvåkning
 - Rutineprøver fra blod
 - Diagnostikk, sykdomsfase
 - Virus vs infeksjon - er fisken virkelig syk?

Anvendelser

- Analyse av sykdomsstatus i familiemateriale
 - Avlskjerne fra felt med CMS (Aqua Gen)
 - Histopat + virus (uført), sykdomsmarkører (pågår)
- Evaluering av funksjonelle fôr på CMS
 - Diett/smitteforsøk i regi av EWOS (start april 2011)
 - Histopat, virus, sykdomsmarkører/mekanismer

Takk for oppmerksomheten!