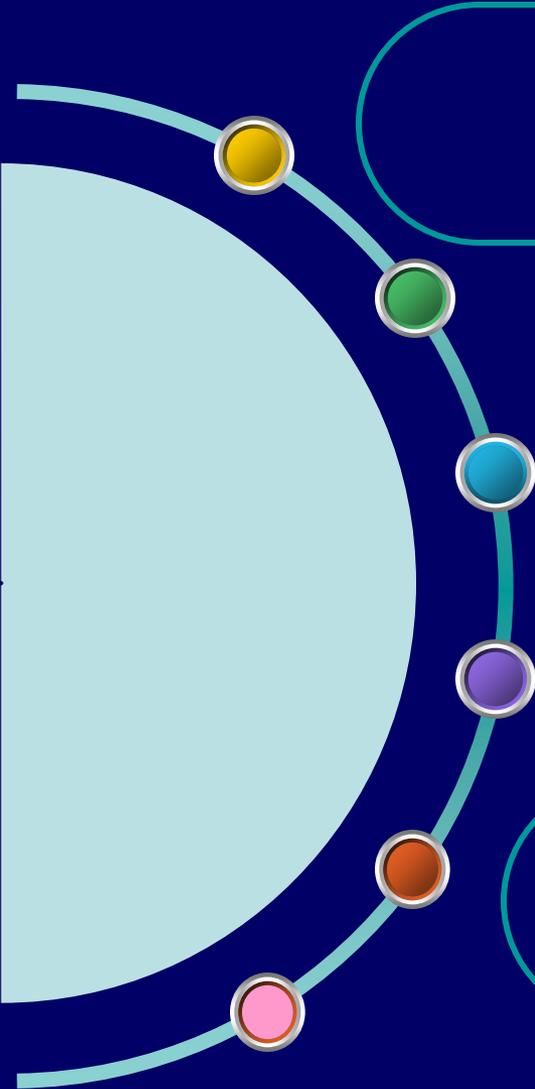


FCM在诊断B淋巴细胞增殖性 疾病中的应用



安徽省立医院中心实验室



FCM检测的基本原理

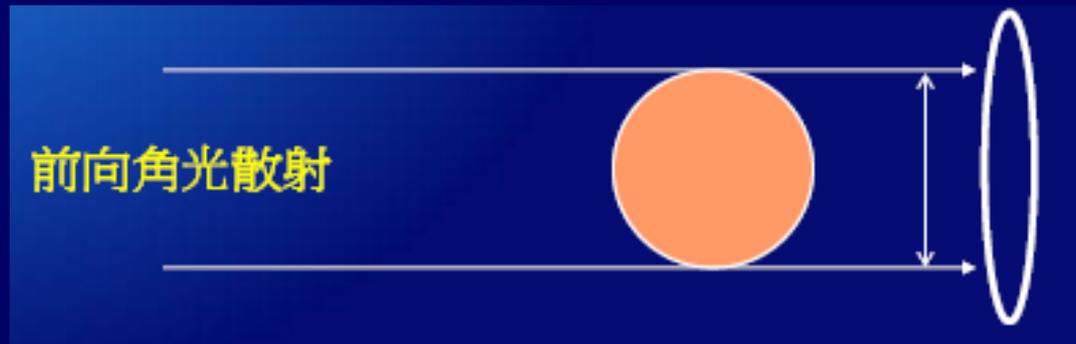
**FCM与病理的相同、
相异之处**

FCM检测B细胞淋巴瘤

一、FCM检测的基本原理



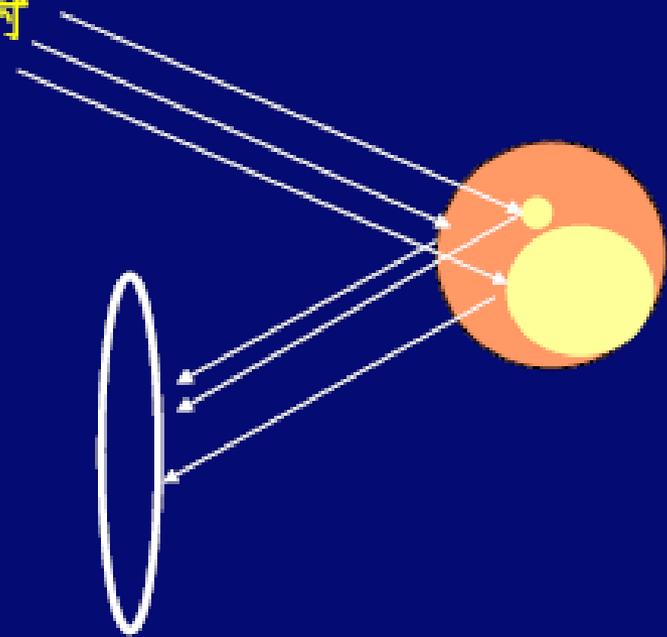
- **FSC (forward scatter):** 前向散射光与细胞大小、表面积相关



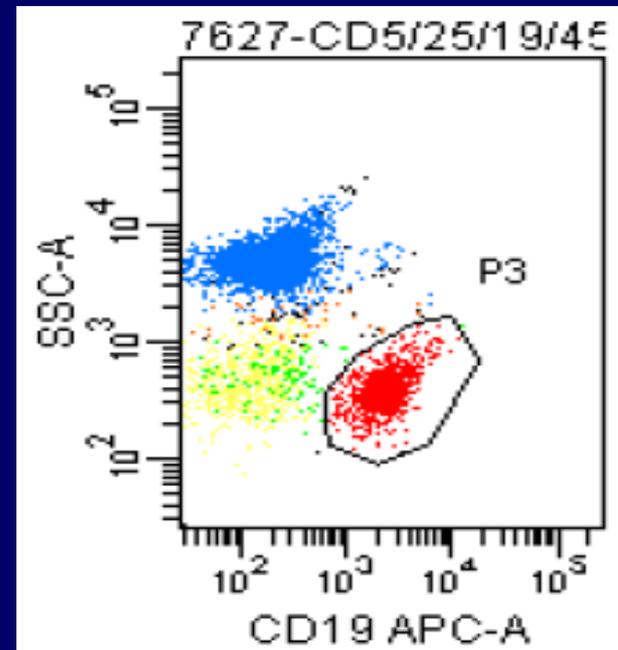
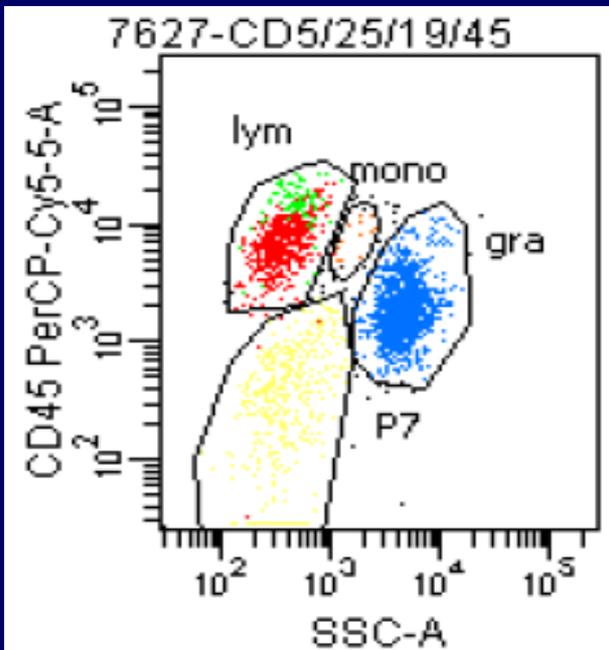
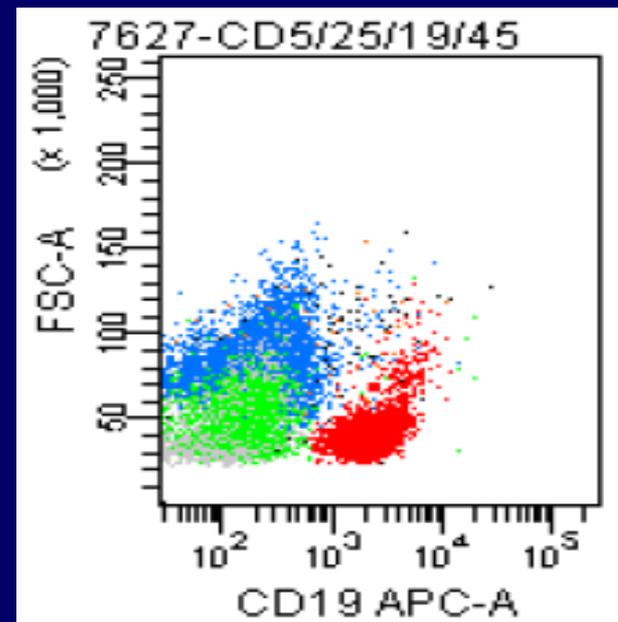
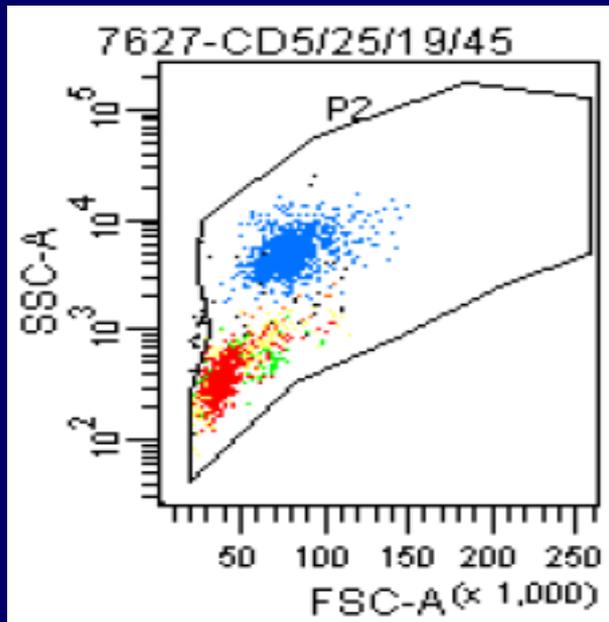
➤ **SSC (side scatter)** : 侧向散射光
反应细胞的颗粒性、结构复杂性

侧向角光散射

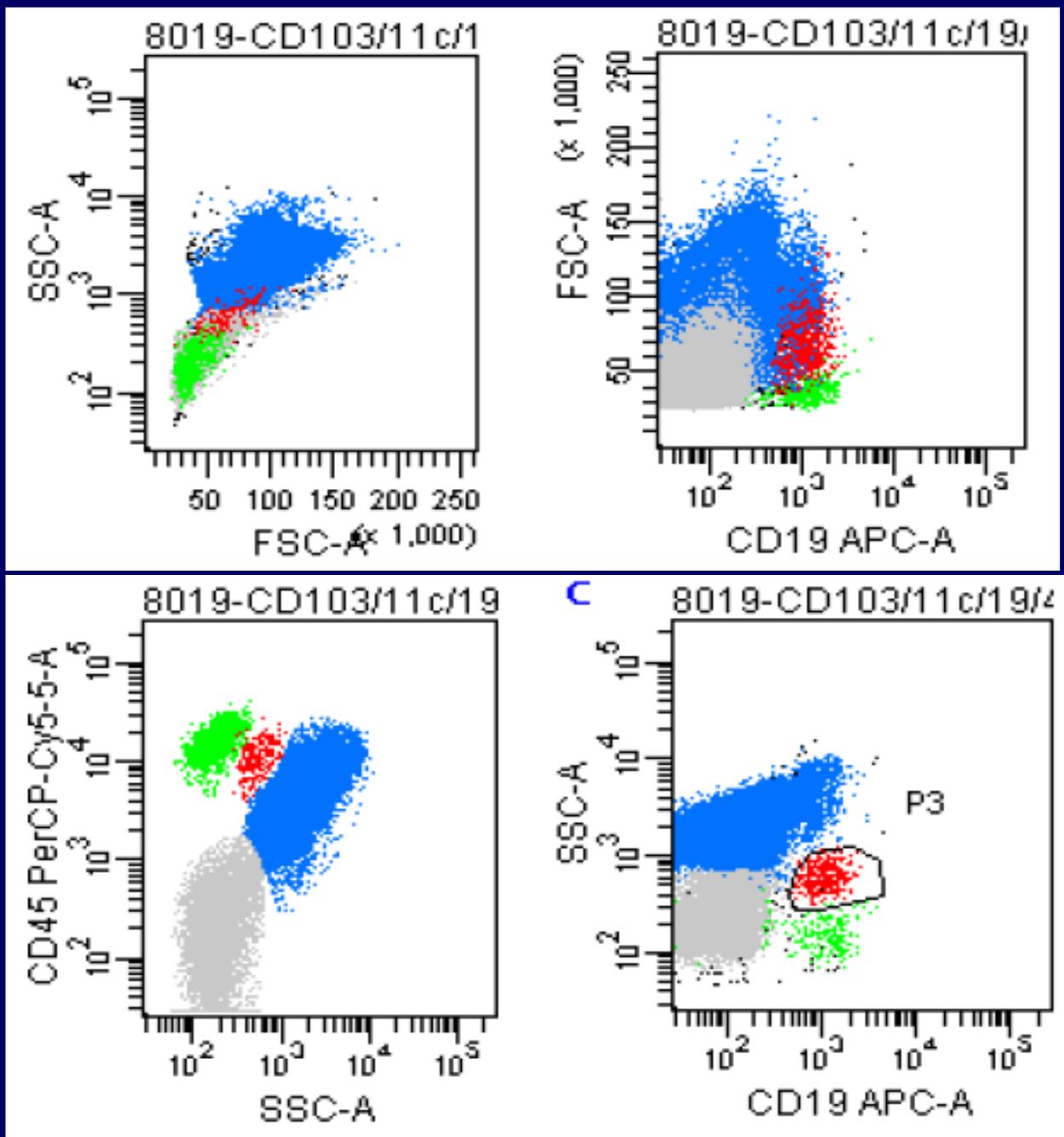
成熟粒细胞>单核细胞>原幼细胞
>淋巴细胞>红细胞



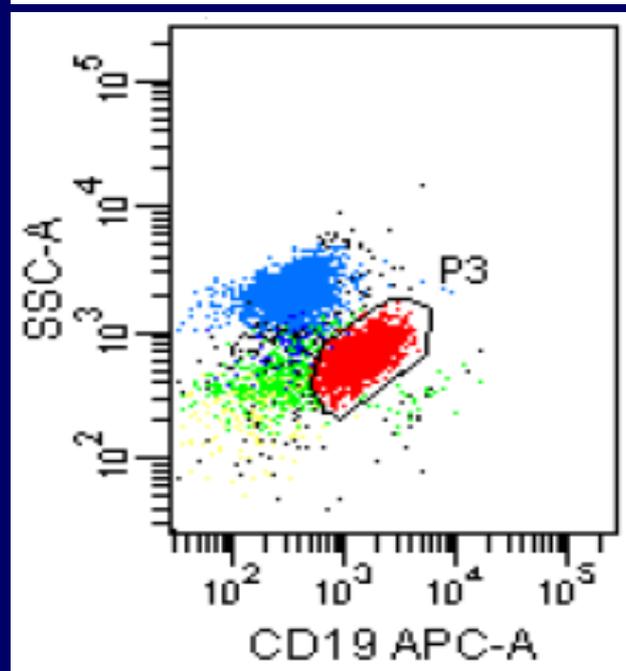
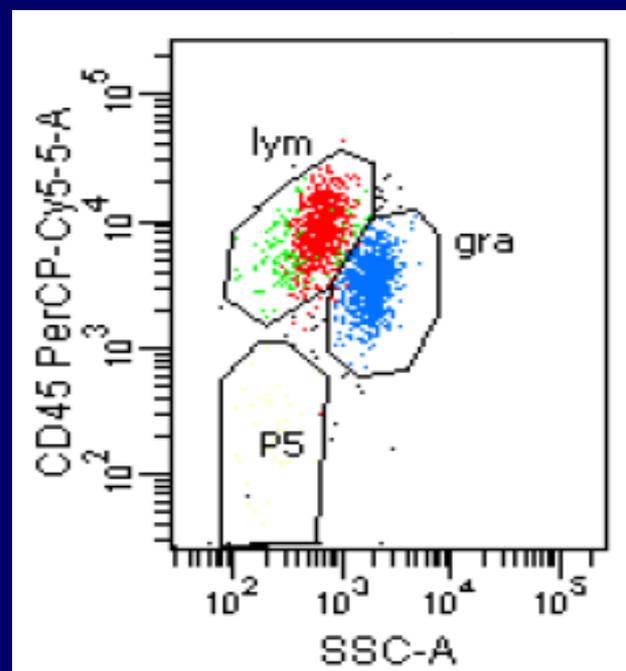
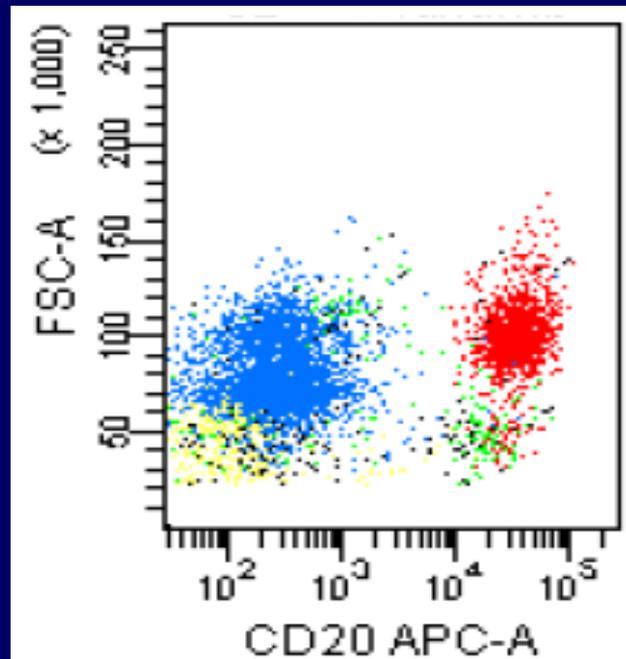
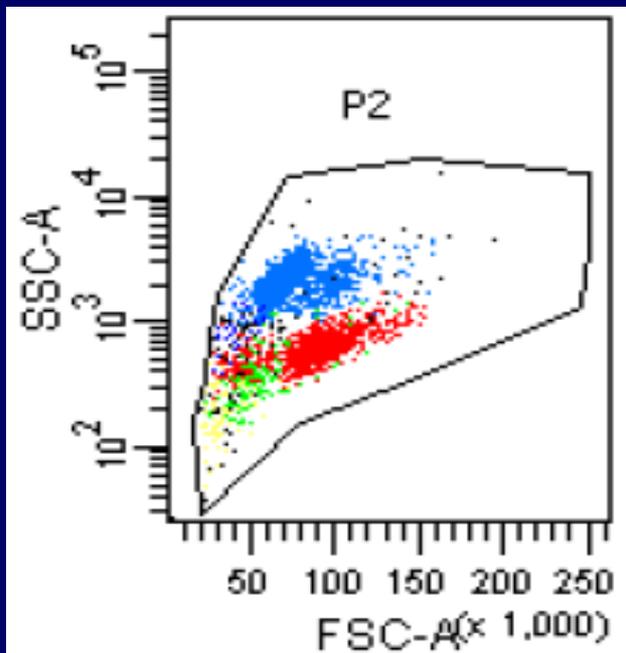
FSC小 SSC小



FSC小到中等,
SSC大



FSC大
SSC大



➤ 适合做流式检测的标本

- 血液
 - 骨髓
 - 各种体液：脑脊液、胸腔积液、腹腔积液
 - 活检组织
 - 细针穿刺物
- } 新鲜标本置于NS或PBS中，不能加固定液！！

二、流式与病理的相同相异之处



➤ 相同之处

- 使用同一诊断标准
- 根据抗体表达情况判断细胞性质---使用相似抗体

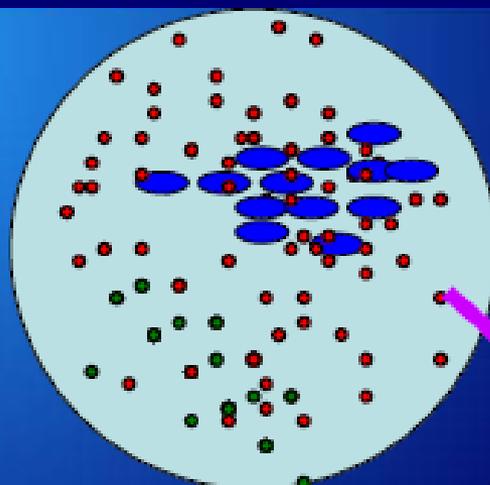
CD分子：CD1-CD363，其中还有很大亚类

非CD分子：如bcl-2,bcl-6,ki 67等，数百种

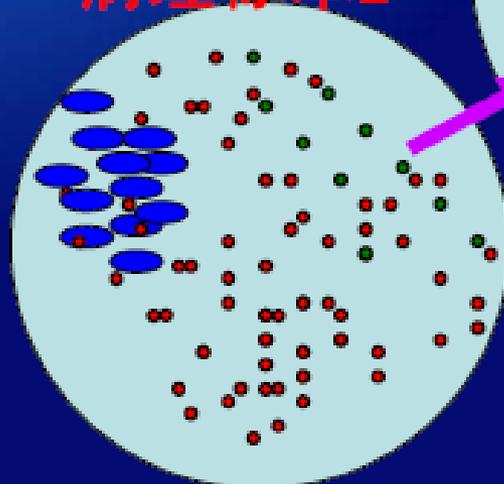
- 各种标本均适合

血液、体液、组织、培养细胞等---所有有活细胞的标本，均可做流式

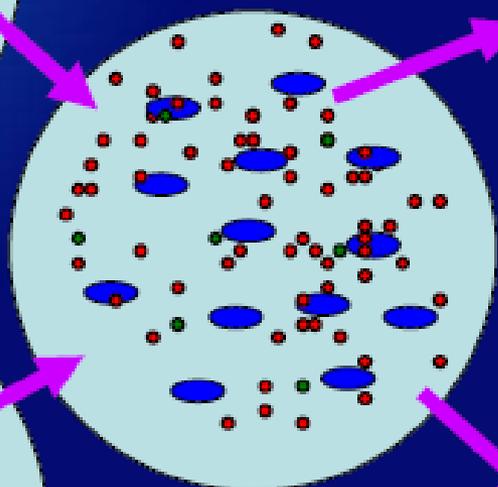
病理标本1



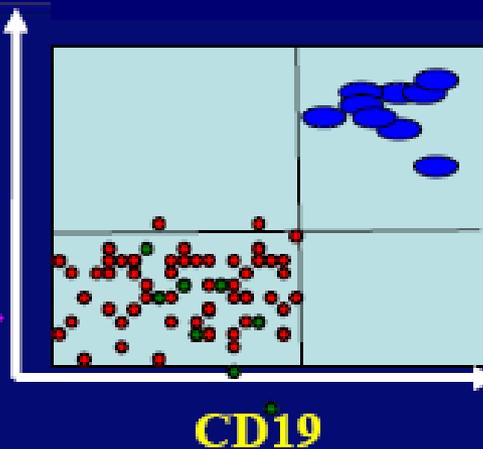
病理标本2



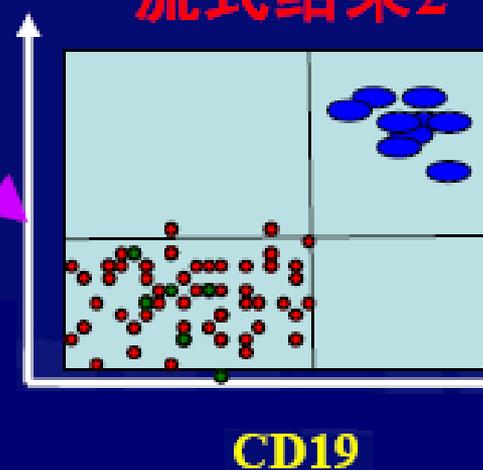
流式处理



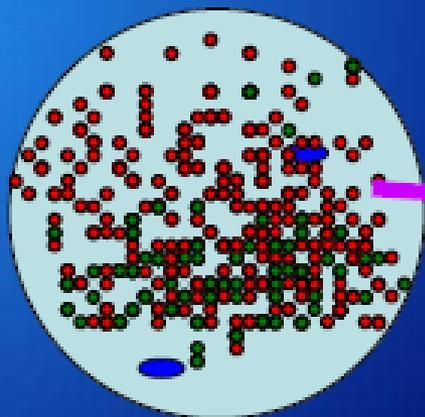
流式结果1



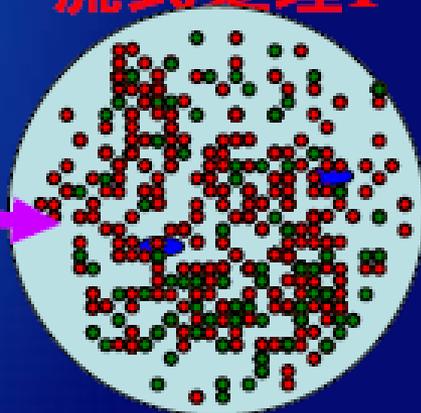
流式结果2



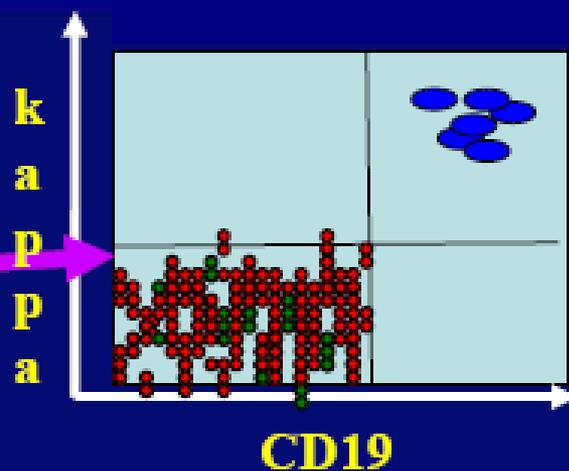
病理标本1



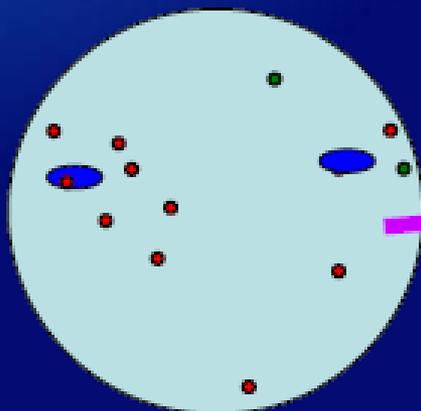
流式处理1



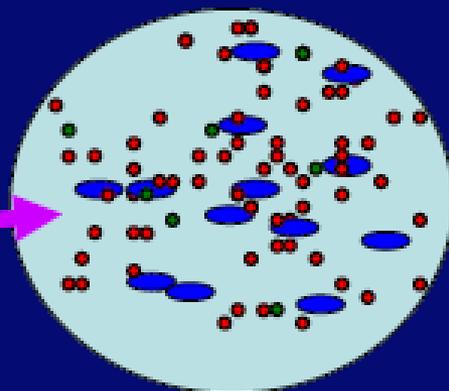
流式结果1



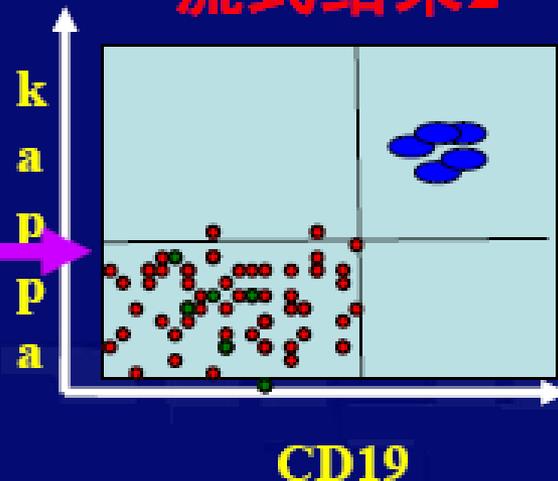
病理标本2



流式处理2

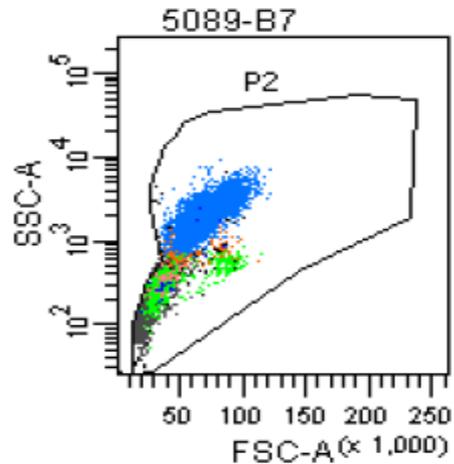


流式结果2

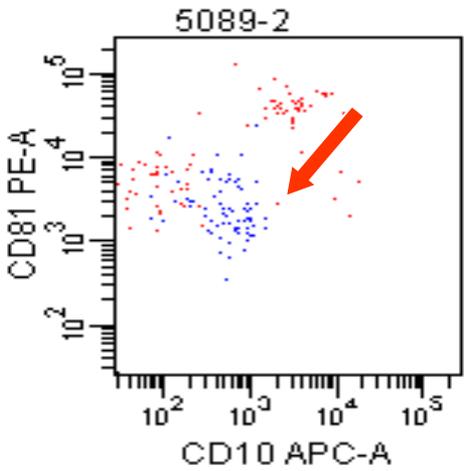
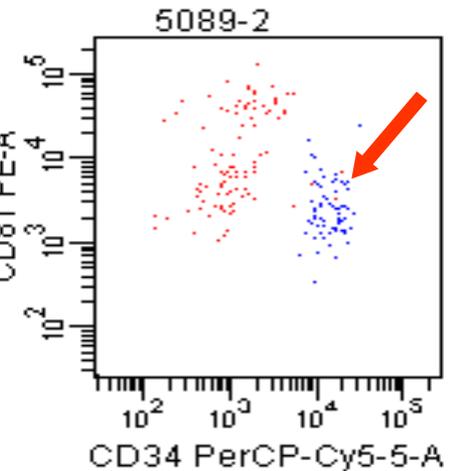
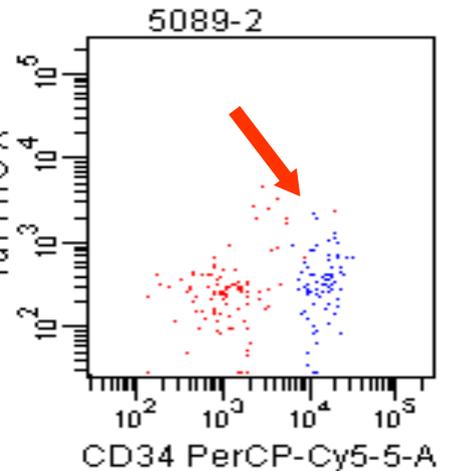
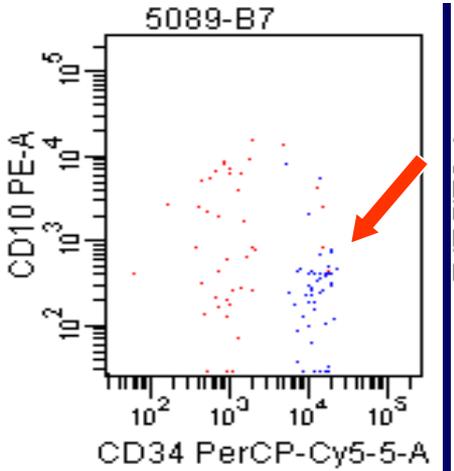
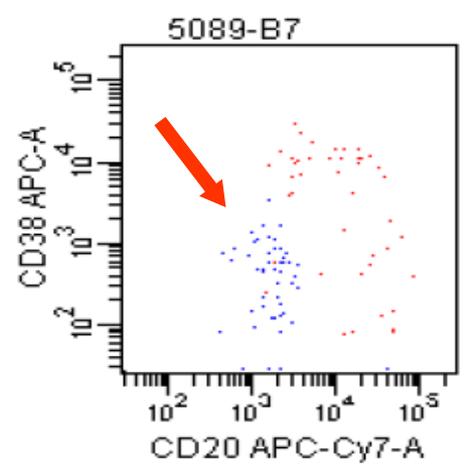
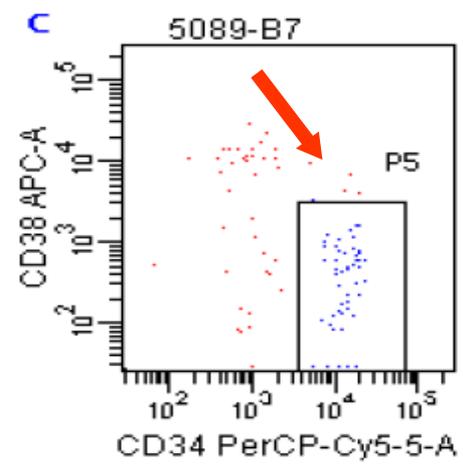
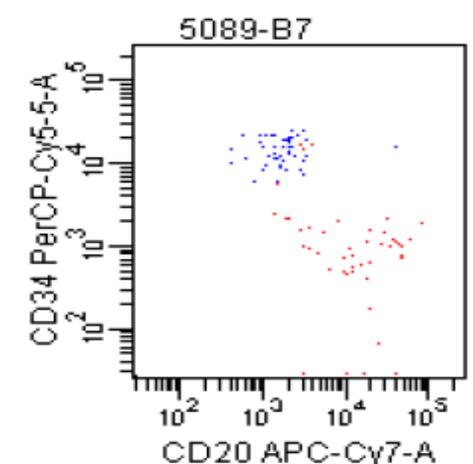
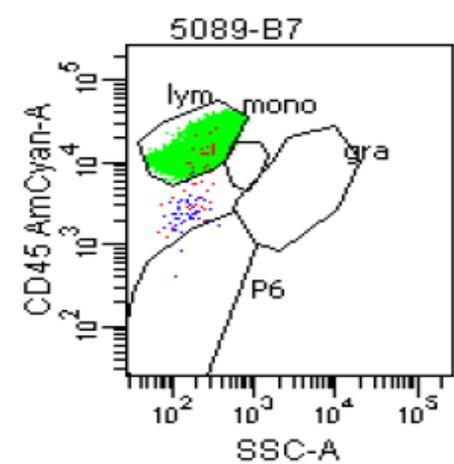
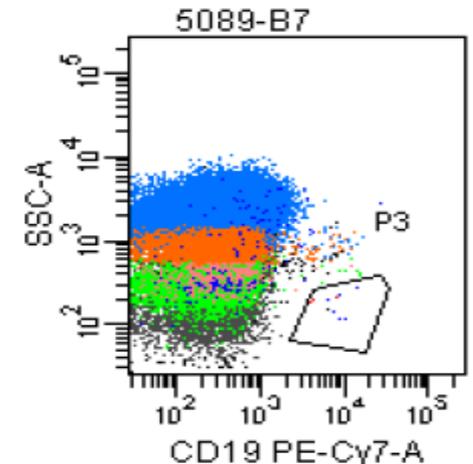
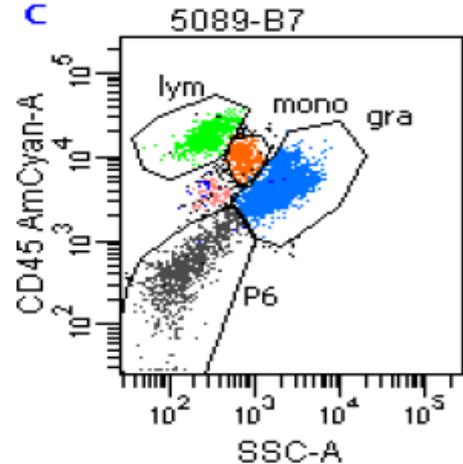


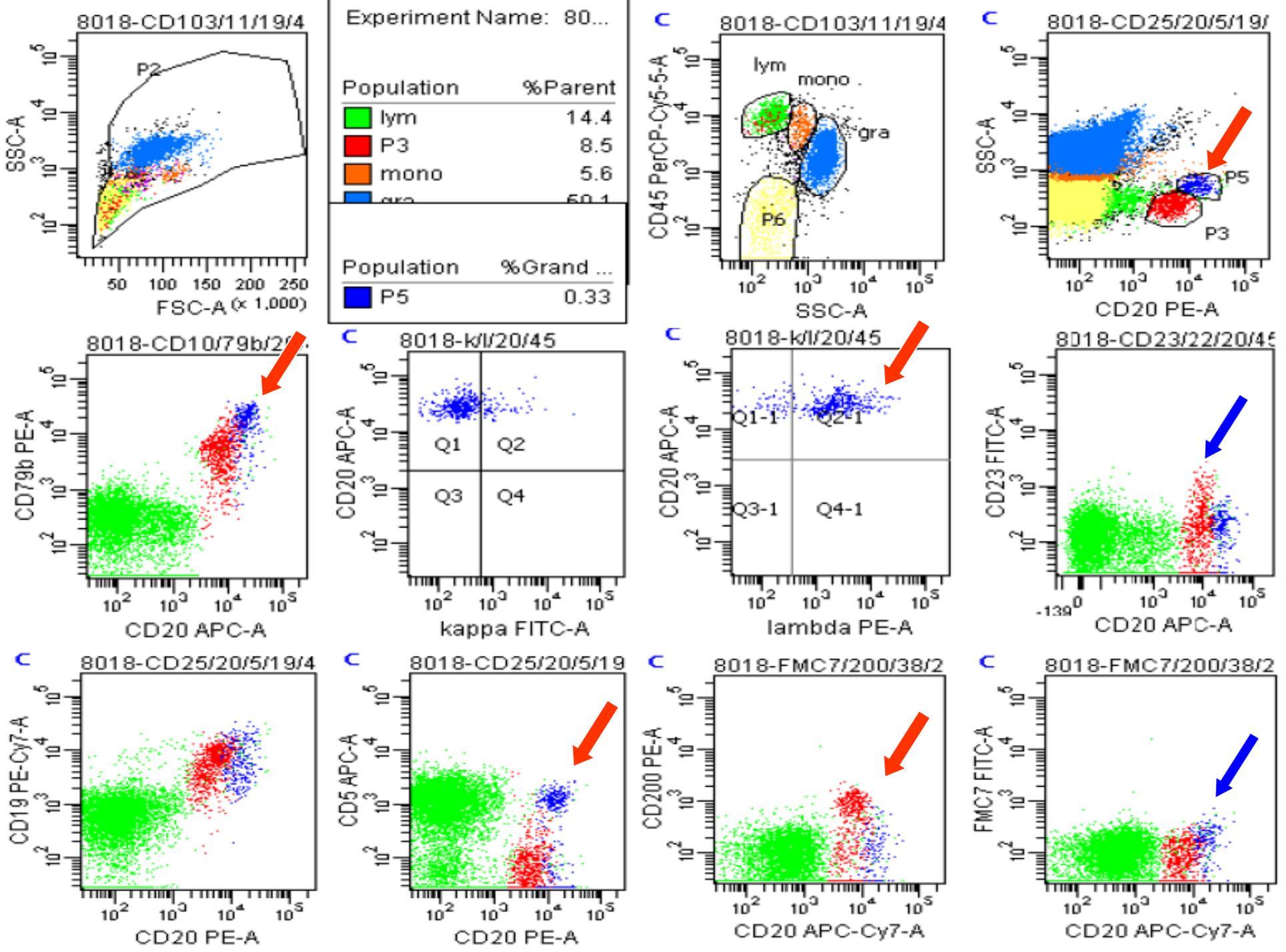
➤ 不同之处---流式特点

- 抗体多、特异性好---定性准确（明确系别、阶段和良恶性）
- 快速获取大量细胞，使用目的抗原设门-----擅长检测比例低（0.01%）、数量少、多种肿瘤细胞并存的情况
- 没有组织结构：对于没有特殊标志的肿瘤，亚型判断困难



Population	%Parent
lym	10.909
mono	5.729
gra	68.975
P6	12.004
P3	0.012
P4	1.031
P5	0.264
P3 AND P5	0.006





三、FCM在诊断B淋巴细胞增殖性 疾病中的应用

➤ B淋巴细胞增殖性疾病 (B-CLPD)

原发白血病:

- 慢性淋巴细胞白血病 (CLL)
- B幼淋巴细胞白血病(B-PLL)
- 毛细胞白血病(HCL)
- 脾B细胞淋巴瘤/白血病，不能分类，包括毛细胞白血病-变异型 (HCL-V)

淋巴瘤白血病期:

- 脾边缘区淋巴瘤(SMZL)
- 套细胞淋巴瘤(MCL)
- 滤泡淋巴瘤(FL)
- 淋巴浆细胞淋巴瘤/华氏巨球蛋白血症(LPL/WM)

➤ B-CLPD共同特征

- 临床特点:

中老年

临床进展缓慢，低度恶性（MCL除外）

可向侵袭性恶性淋巴瘤转化

治疗后可缓解，但难以治愈

- 形态学:

成熟小淋巴细胞为主，部分可出现中等大小淋巴细胞

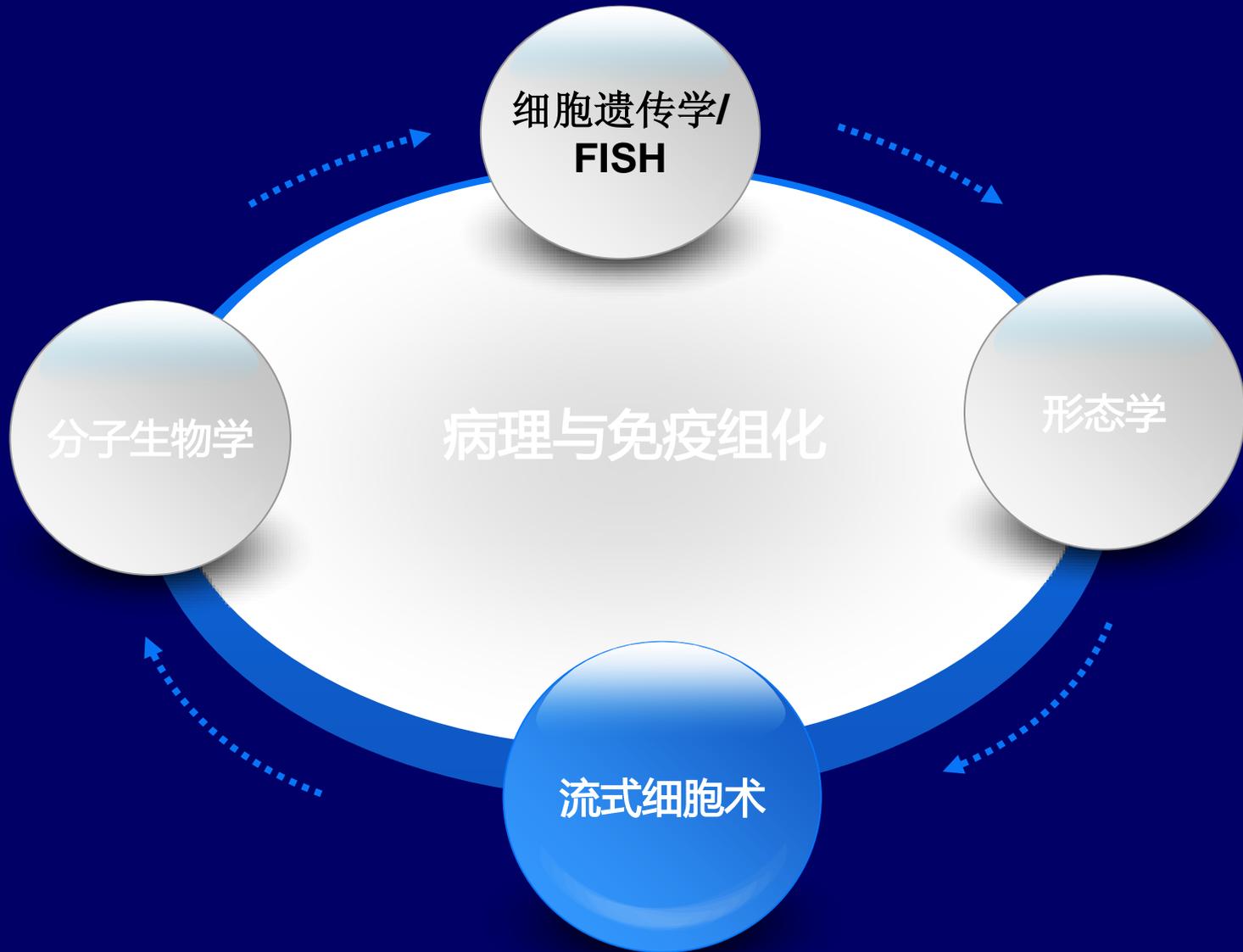
- 免疫表型:

表达B细胞相关抗原和免疫球蛋白单一轻链

- 基因重排:

免疫球蛋白重链（IgH）或/和轻链（IgL）基因重排

➤ 淋巴瘤的诊断方法



➤ FCM确立恶性细胞的依据

成熟淋巴细胞的单克隆性:

- ◆ B细胞单克隆的胞膜Ig轻链
- ◆ 浆细胞单克隆的胞浆Ig轻链
- ◆ NK细胞的KIR
- ◆ T细胞的TCR Vbeta

➤ 免疫球蛋白轻链限制性:

- 单克隆B细胞
 - ◆ Kappa: Lambda < 0.3:1 或 > 1:3
 - ◆ 成熟B细胞不表达膜免疫球蛋白
- 多克隆正常B可能会掩盖少量单克隆性B细胞
- 精确设门很重要
- 单克隆 ≠ 肿瘤

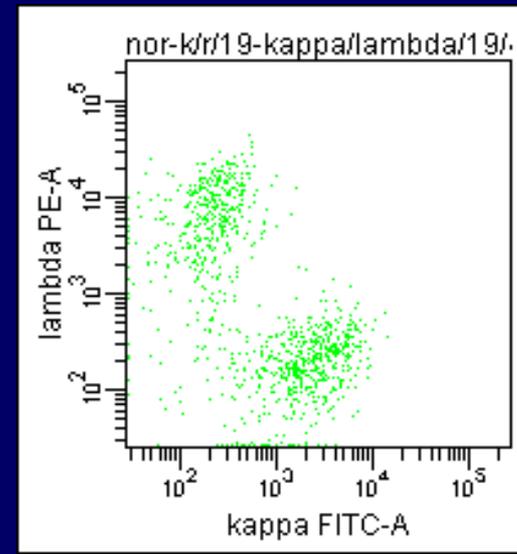
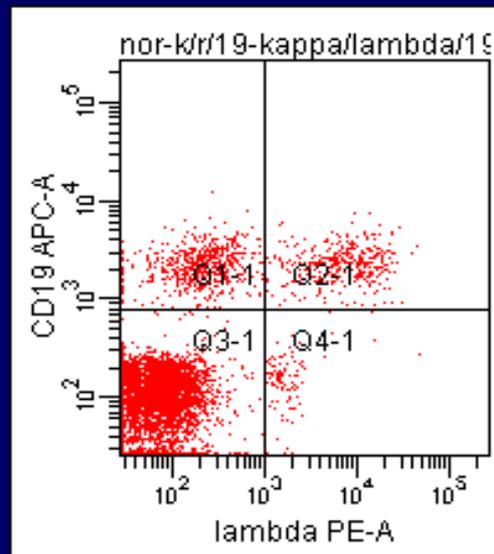
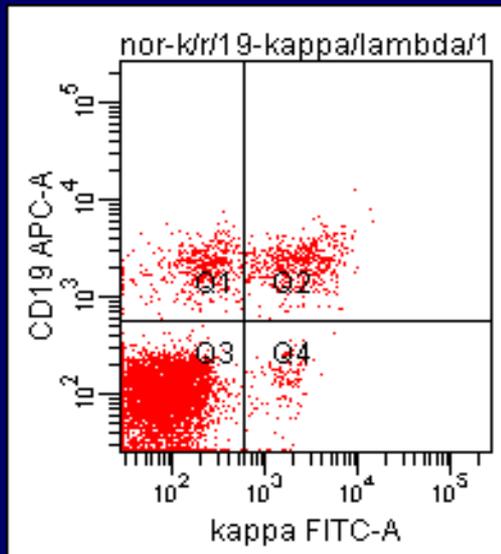
▶ 淋巴瘤与白血病免疫分型不同之处

	白血病	淋巴瘤
累及骨髓比例	> 20%	0.01%~90%
同系疾病	表达相似	表达差别大
亚型	少	多
设门	CD45/SSC	多样化
正常细胞干扰	少	多
需要抗体	少	多

淋巴瘤检测困难：低比例；多样化；标本稀释、肿瘤细胞脆弱增加检测难度

▶ B淋巴细胞在正常骨髓、外周血及淋巴结中的免疫表型

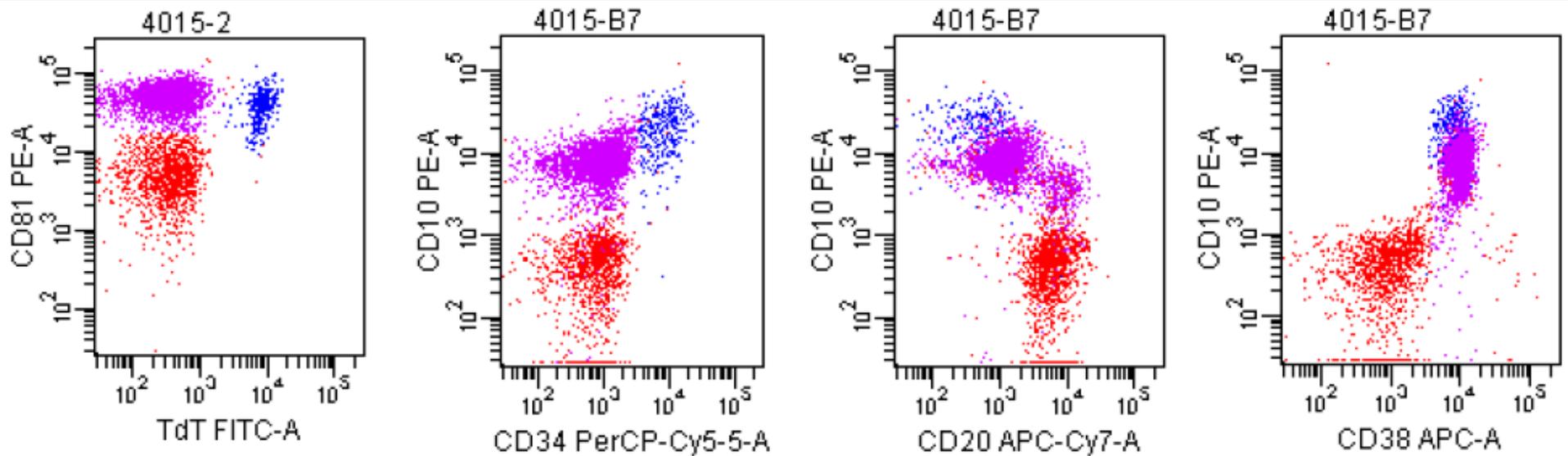
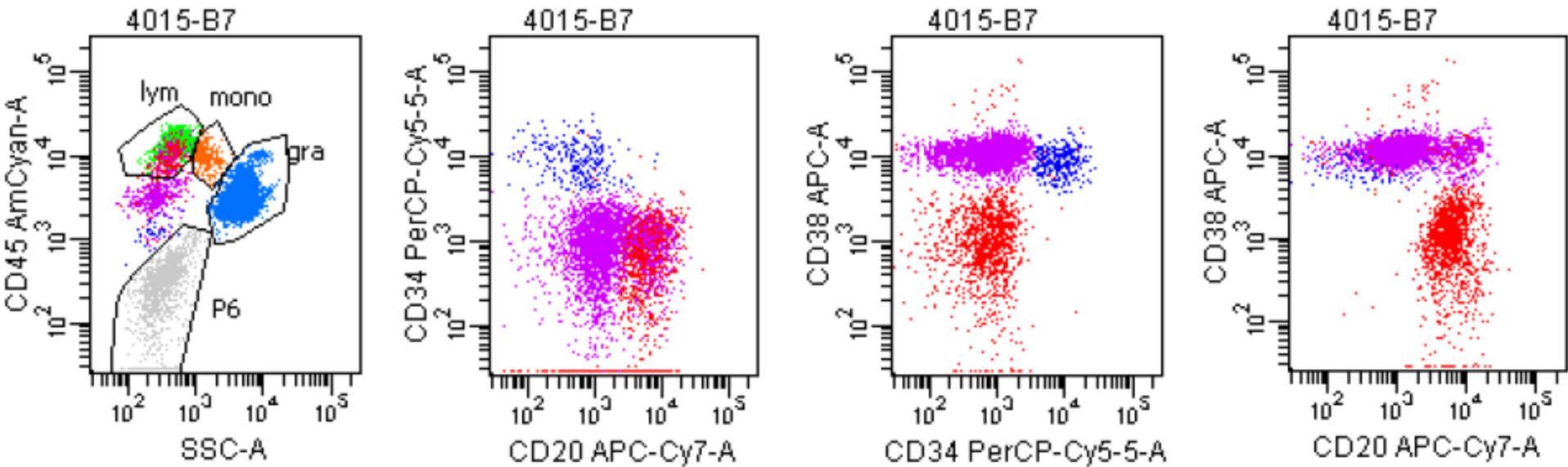
• 外周血

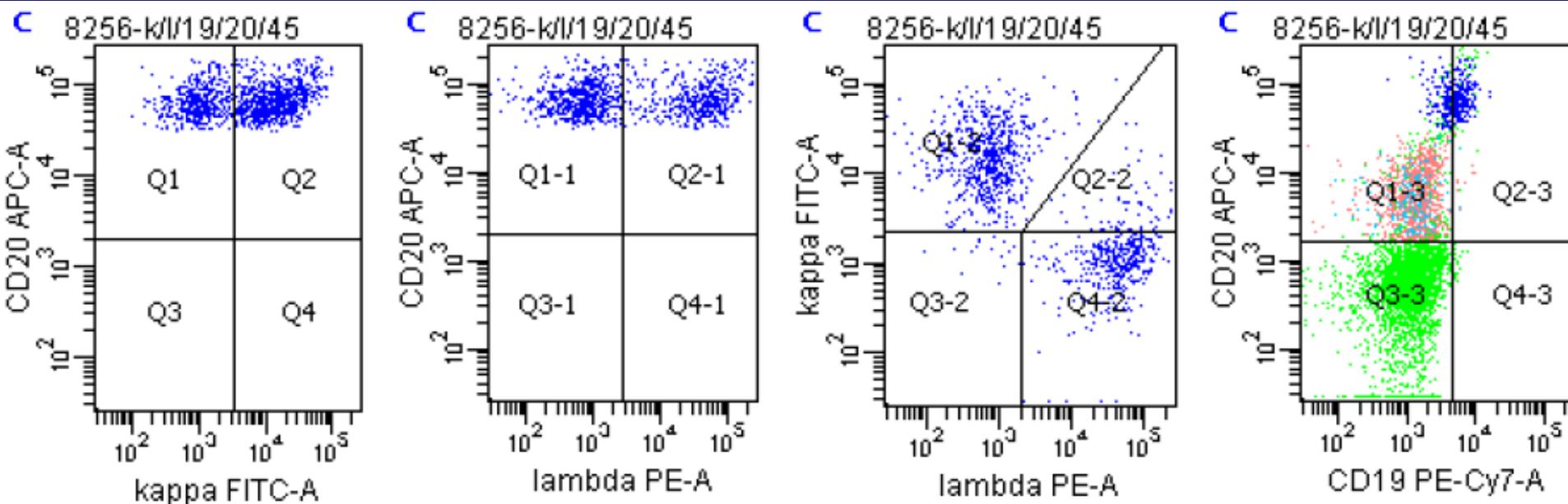
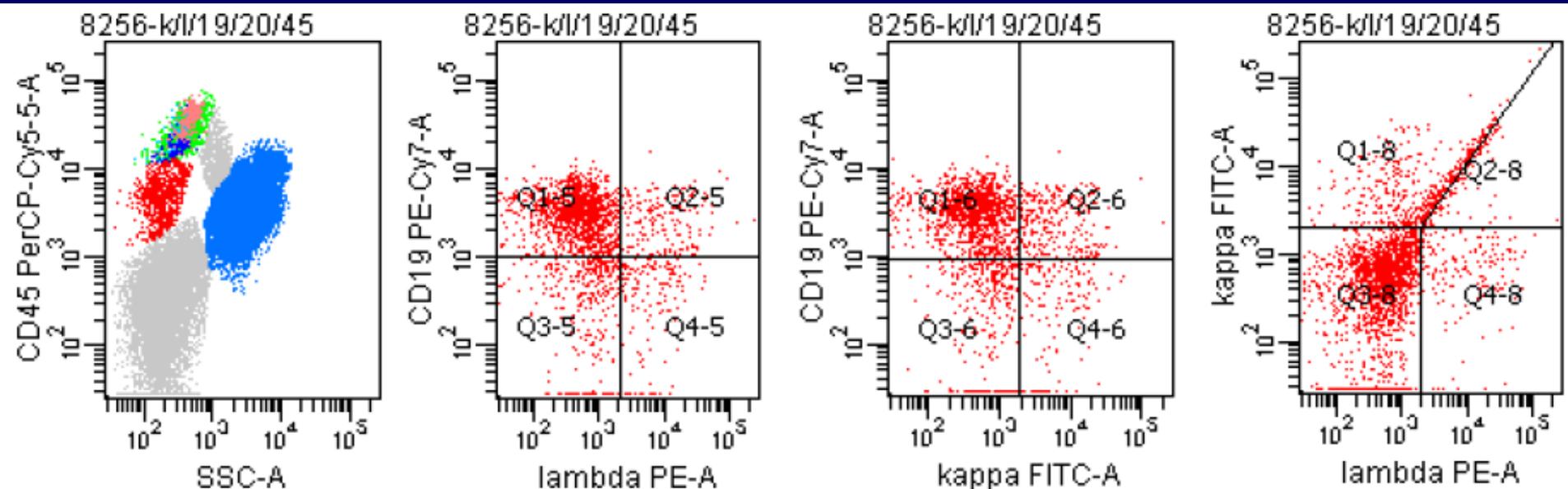


淋巴细胞设门

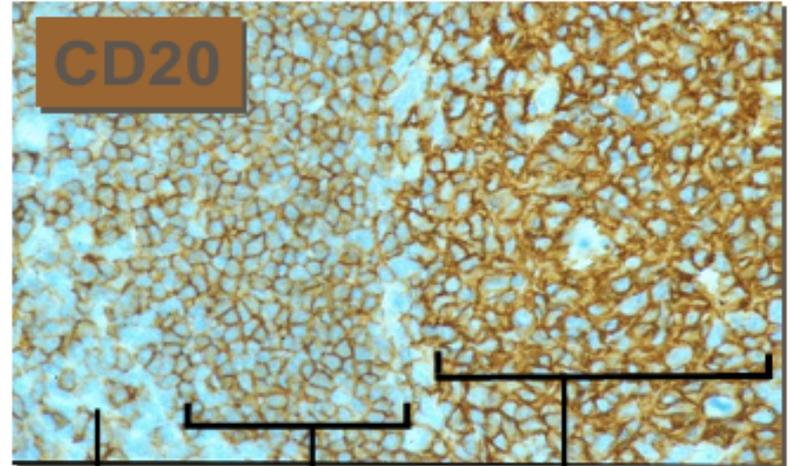
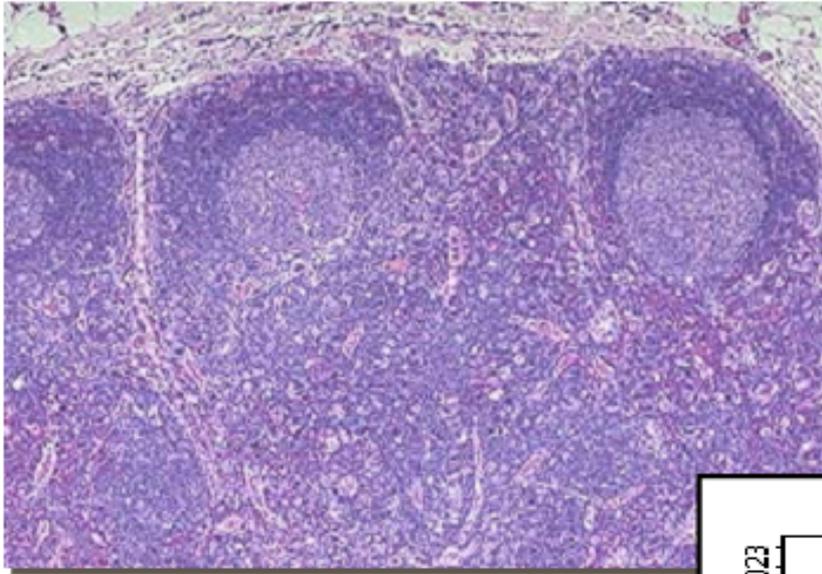
CD19+细胞设门

• 正常骨髓



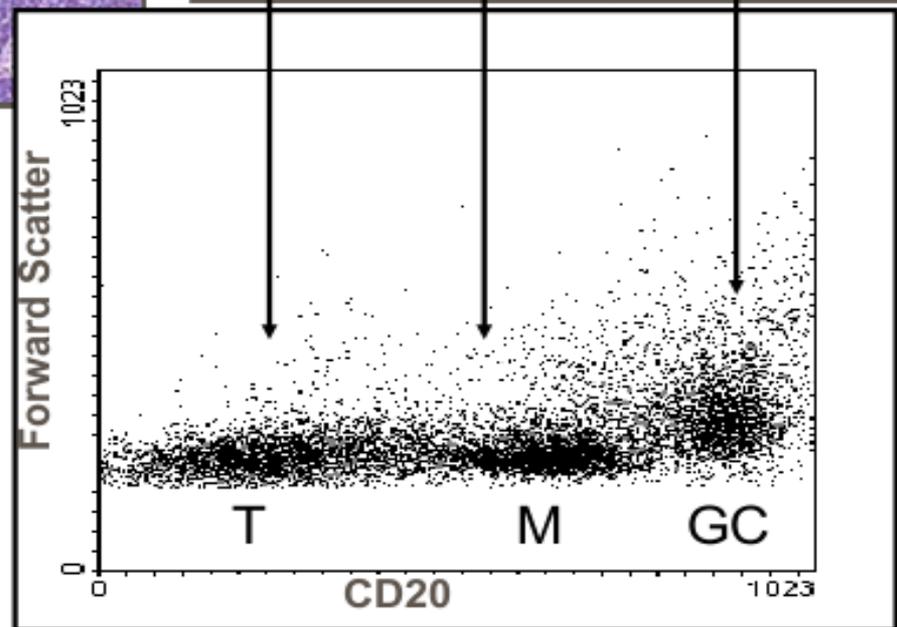


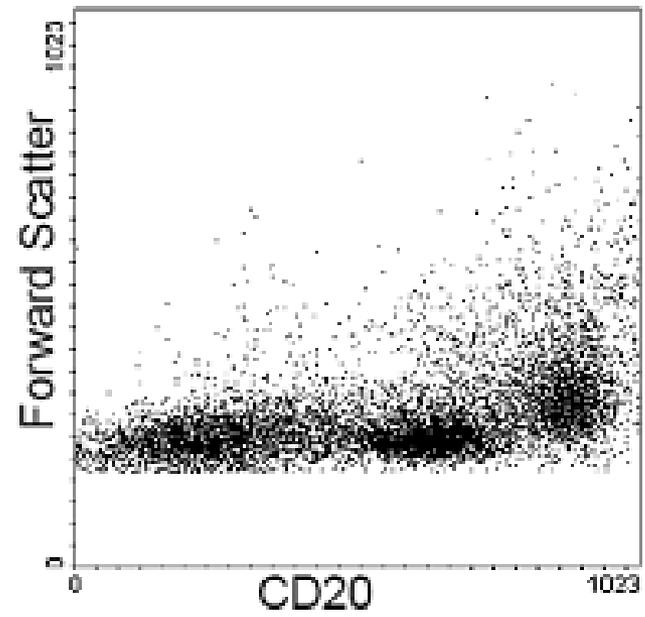
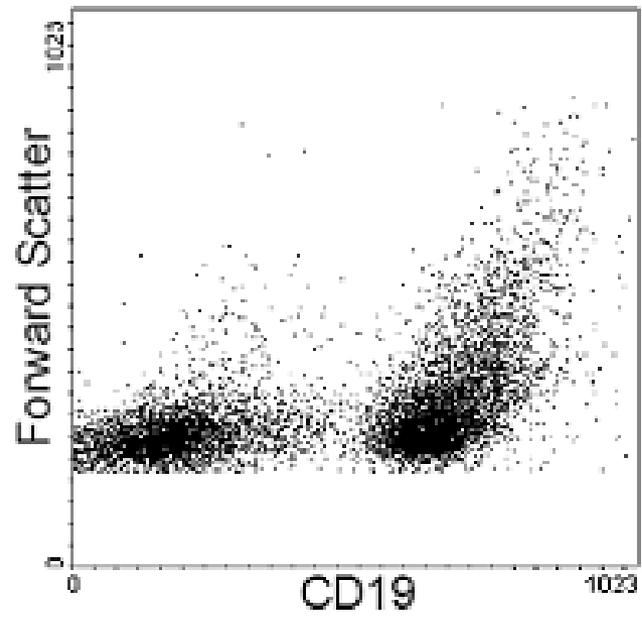
• 淋巴结



Reactive Lymph Node

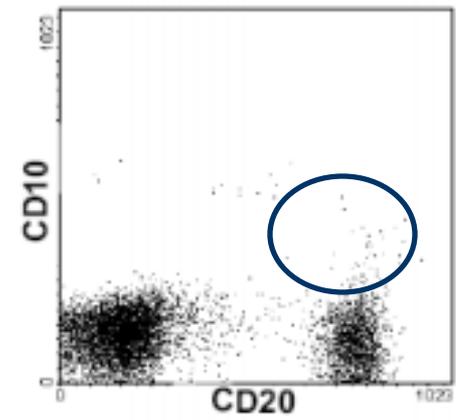
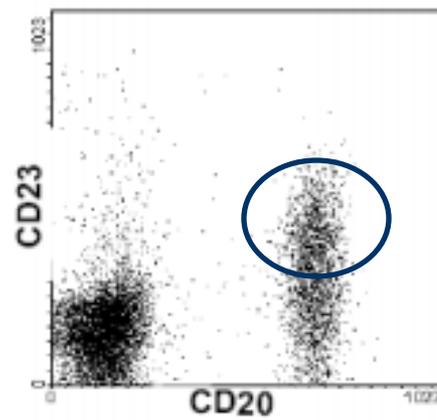
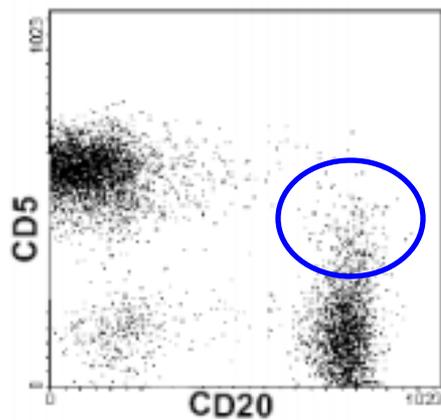
T T-Cells
M Mantle Cells
GC Germinal Center Cells



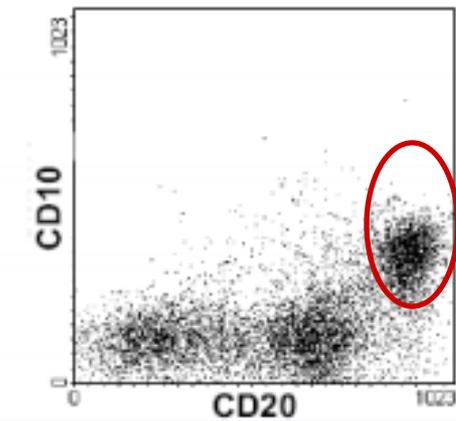
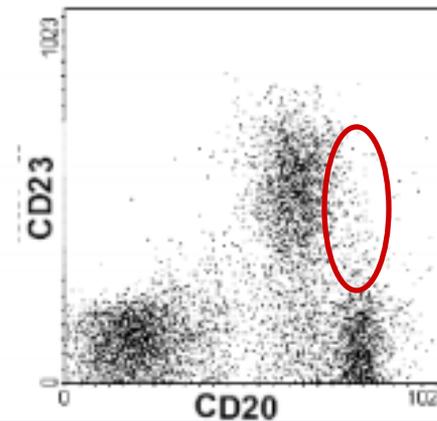
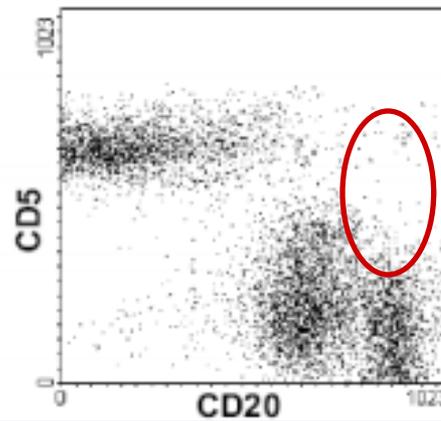


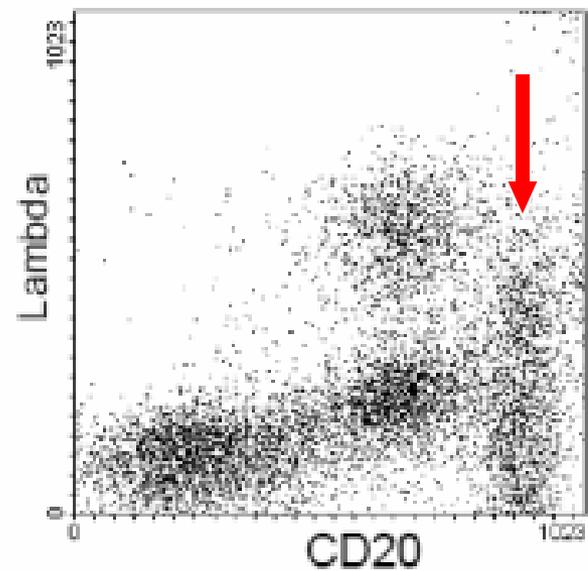
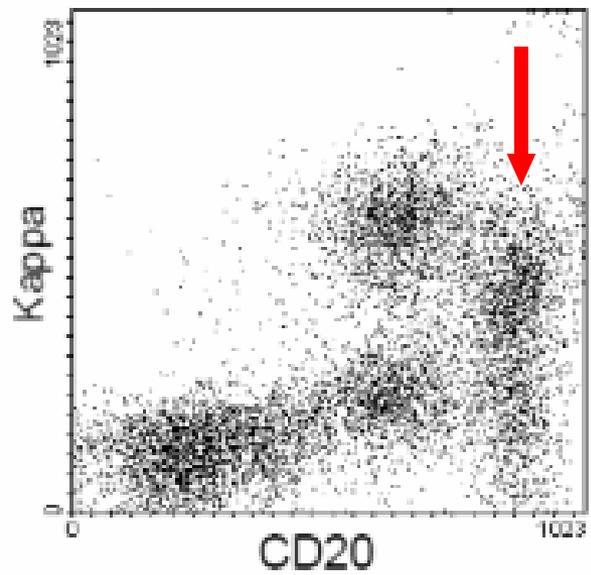
CD5, CD23和CD10在外周血和淋巴结中的表达特点

外周血



淋巴结

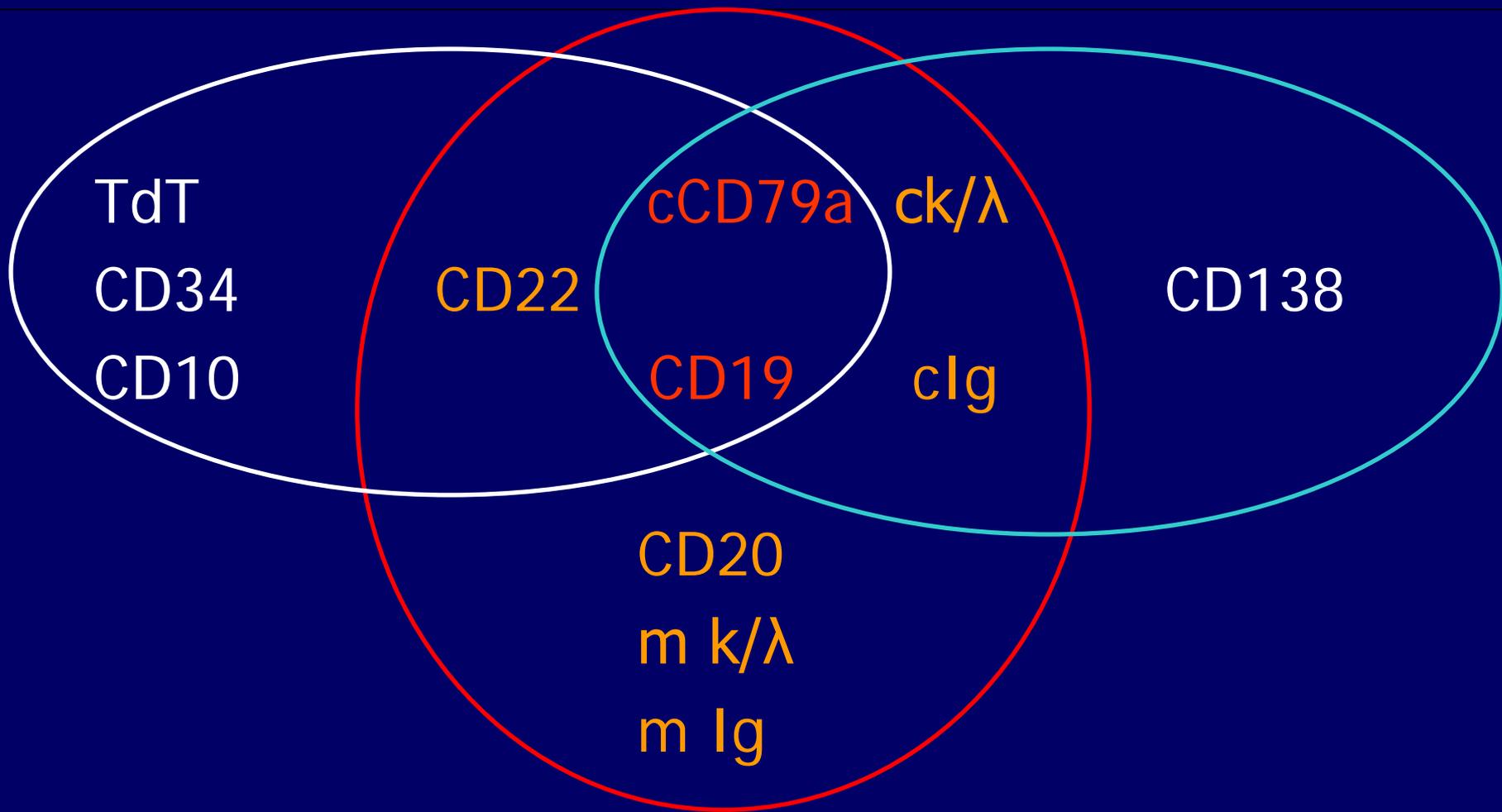




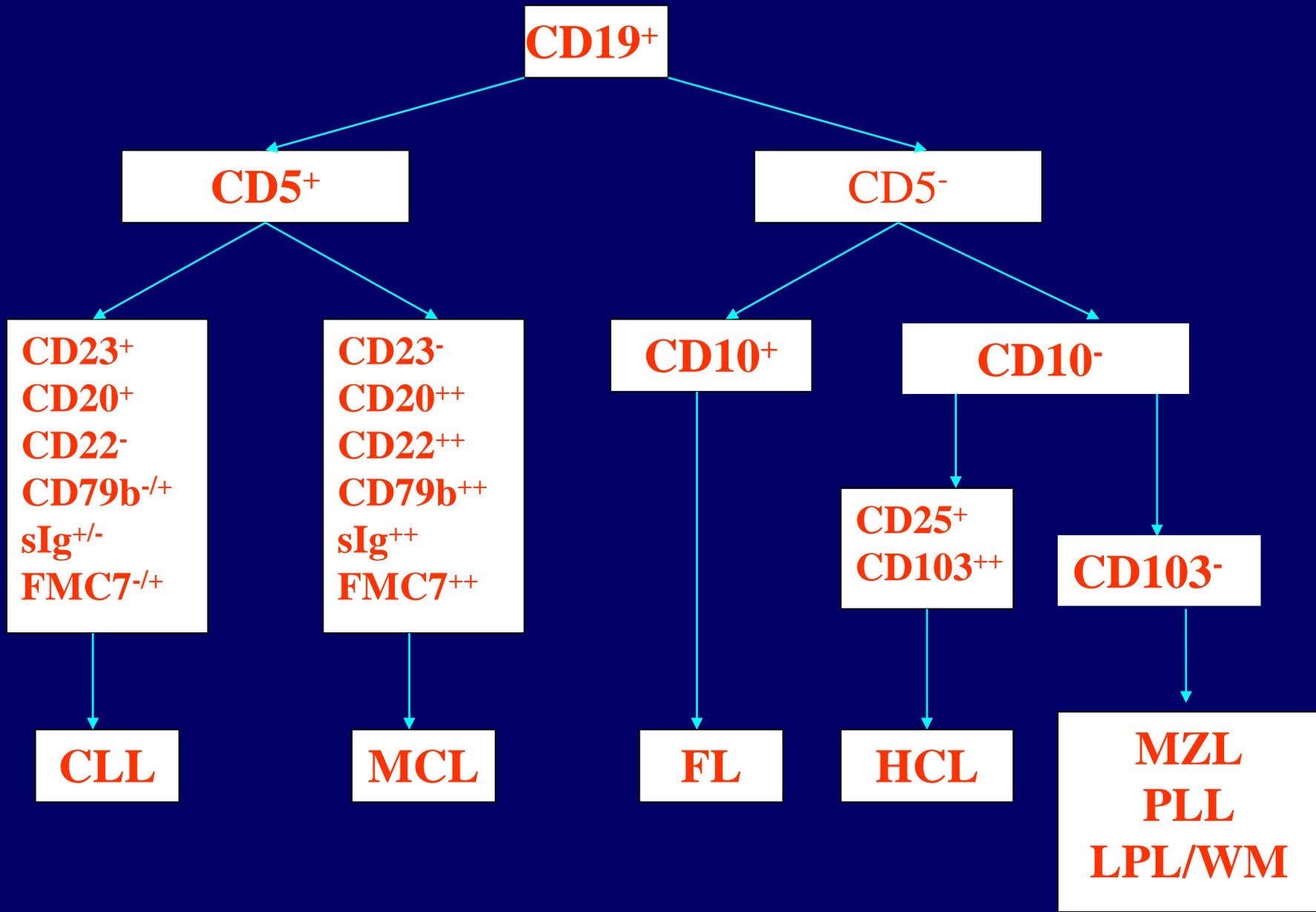
幼稚B细胞

成熟B细胞

浆细胞

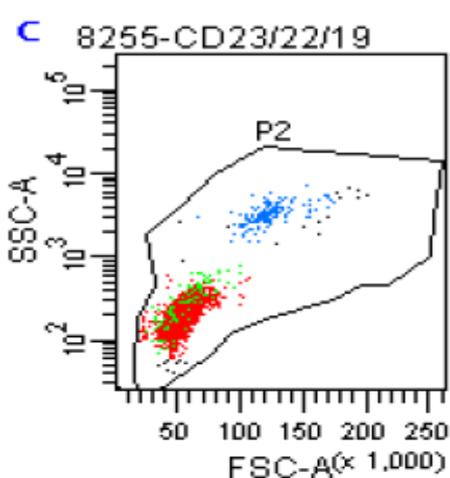


➤ B-CLPD的诊断和鉴别诊断



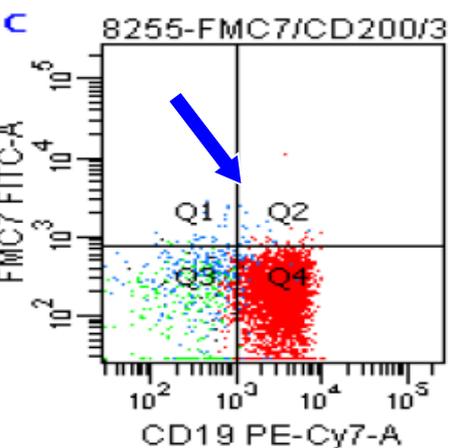
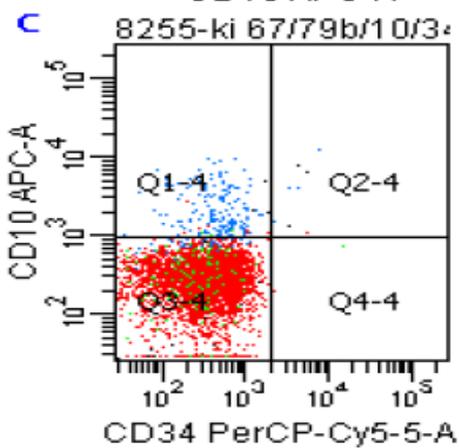
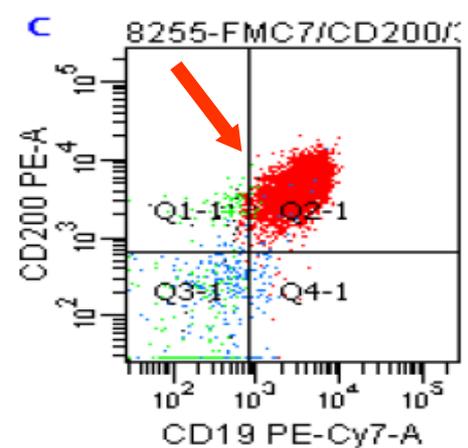
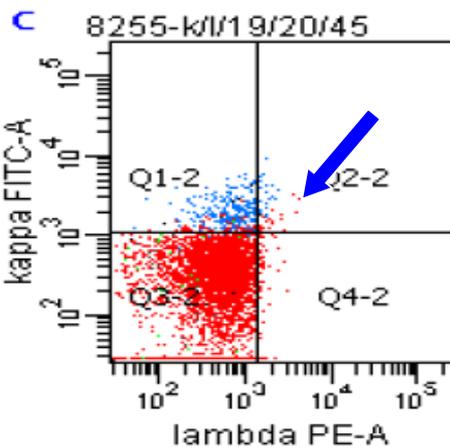
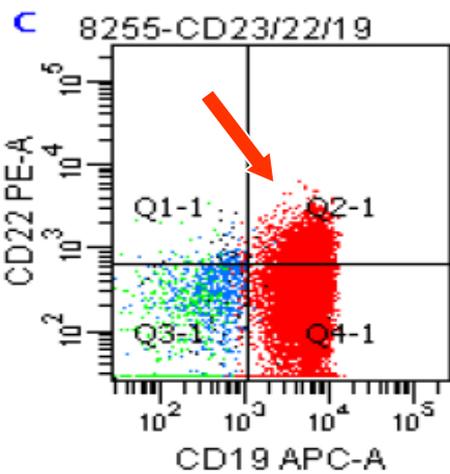
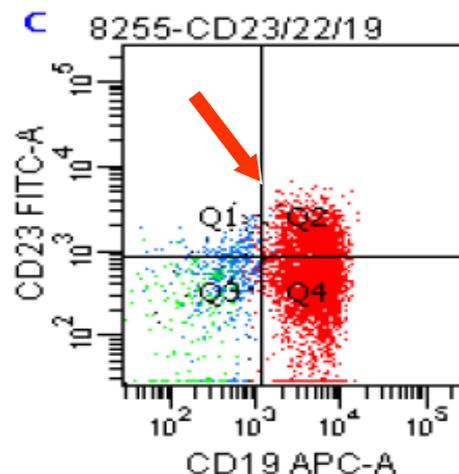
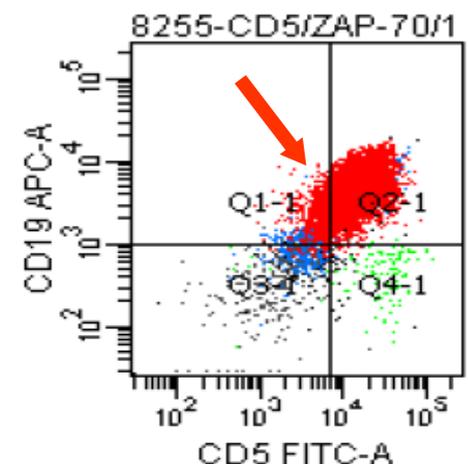
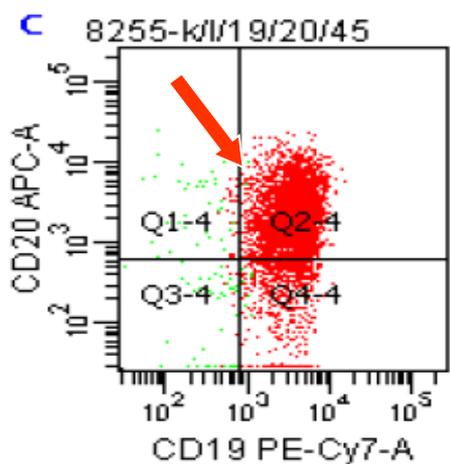
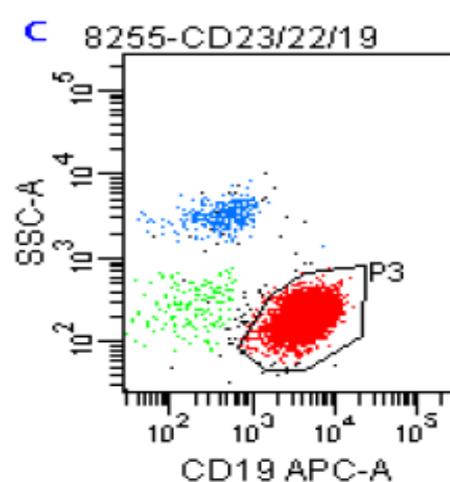
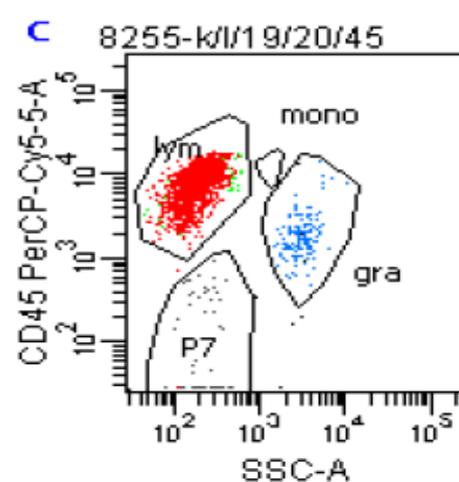
➤ CLL免疫表型积分

	CLL	积分	其他B-NHL	积分
SIg	弱阳性	1	强阳性	0
CD5	+	1	- (除了MCL)	0
CD23	+	1	-	0
CD79b/CD22	弱阳性	1	强阳性	0
FMC7	-	1	+	0
	CLL: 4~5分		其他B-NHL: 0~2分	



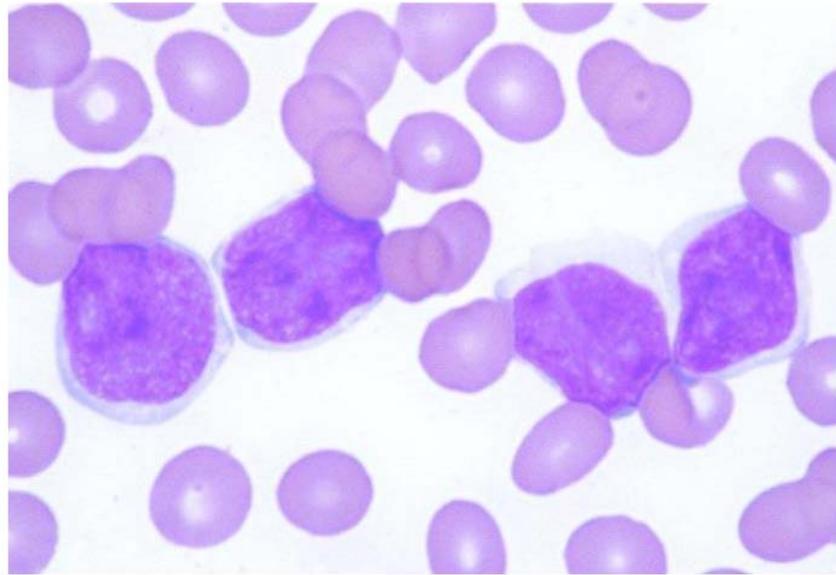
Experiment Name: 82...

Population	%Parent
P2	98.8
lym	91.4
mono	0.0
gra	6.1
P7	2.1
P3	89.1

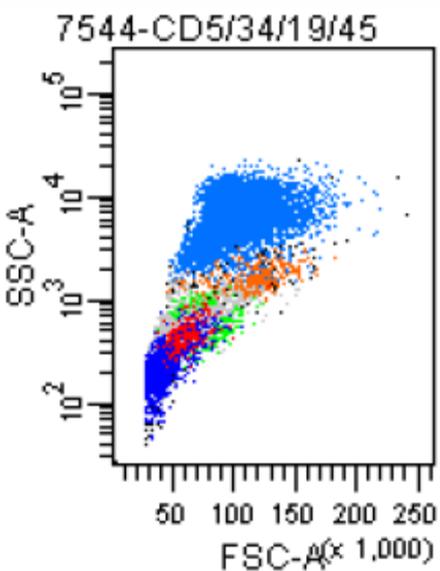


➤ MCL

特征性的染色体异常t(11;14)(q13;q32);
CCND1阳性

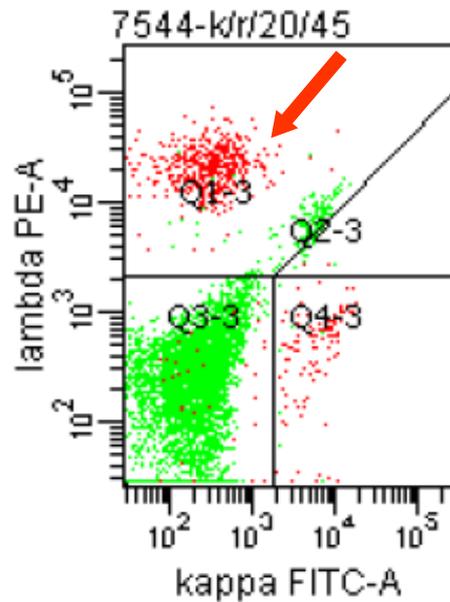
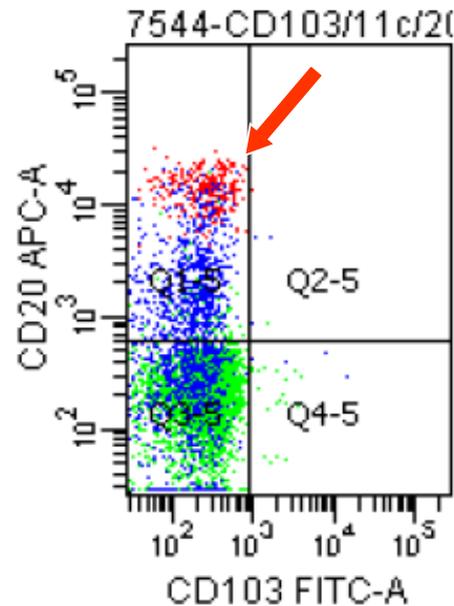
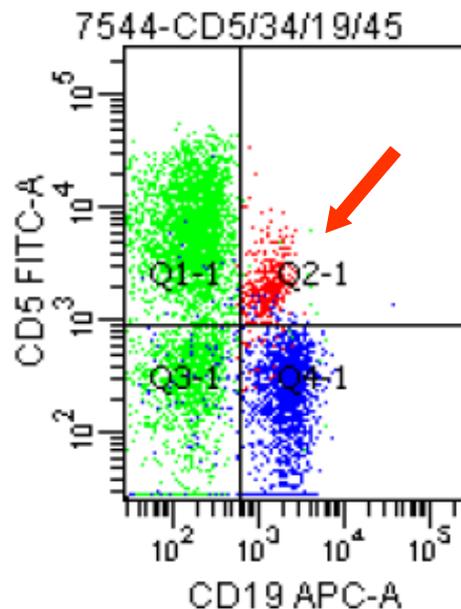
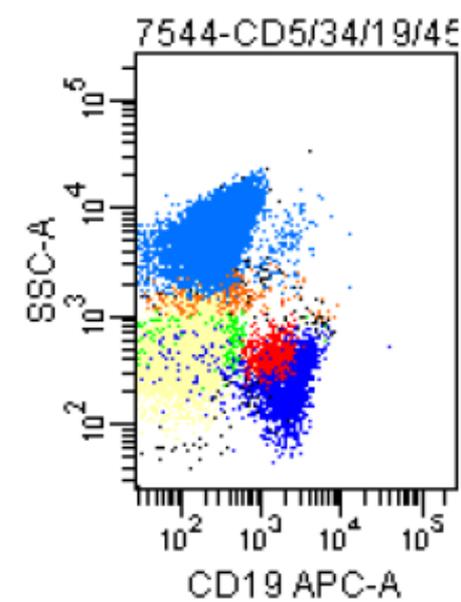
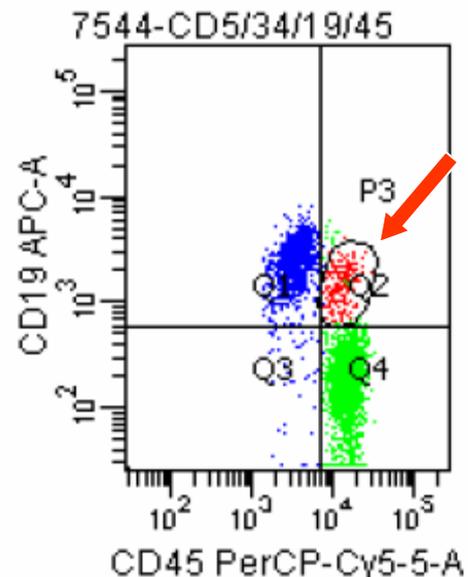
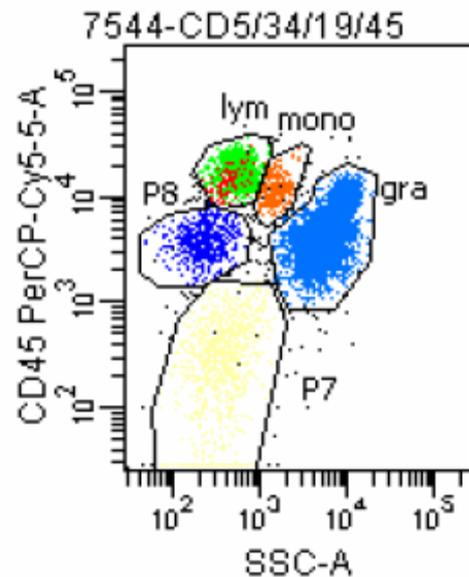


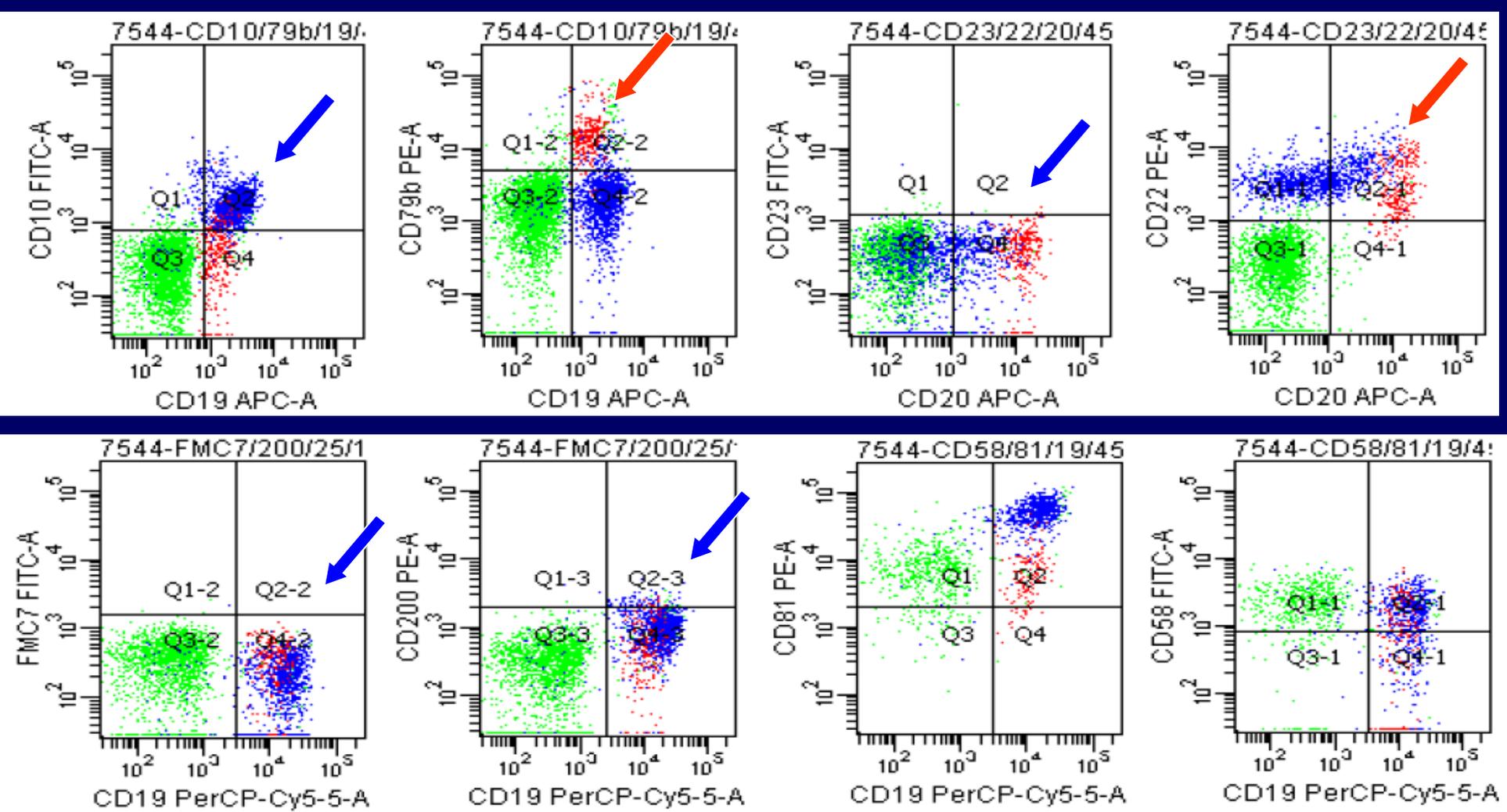
Small to medium size cells slightly irregular nuclear contour -
like mantle cell lymphoma



Experiment Name: 75...

Population	%Parent
P2	95.0
lym	15.2
P3	8.7
mono	3.2
gra	60.2
P7	13.2
P8	6.1

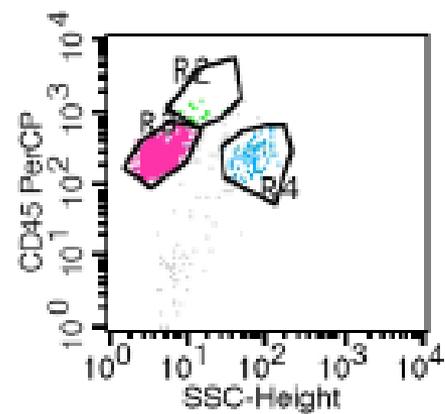




结论：发现1.4%（占全部细胞）的CD5+CD23-CD10-成熟小B细胞，考虑B-CLPD，建议进一步FISH检测t(11;14)。

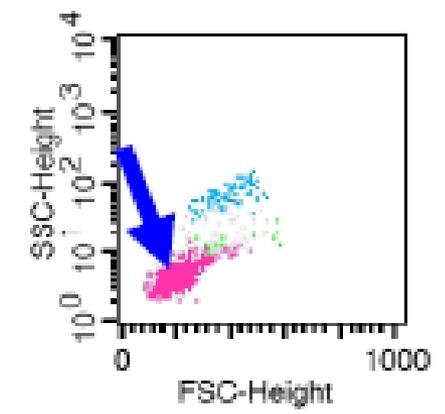
➤ 滤泡淋巴瘤(FL)

- 来源：生发中心B细胞
- 免疫表型特点：
 - ◆ 生发中心抗原:CD10、BCL6、BCL2;
CD10: FL<DLBCL<BL
 - ◆ CD20荧光强度强于正常淋巴细胞

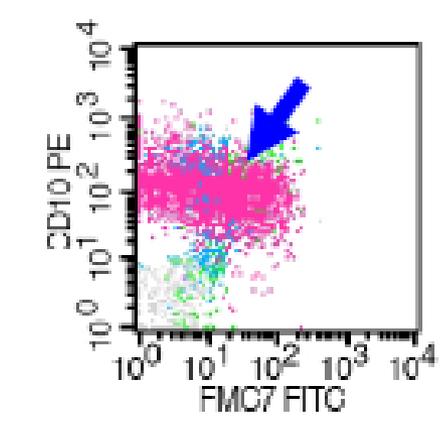
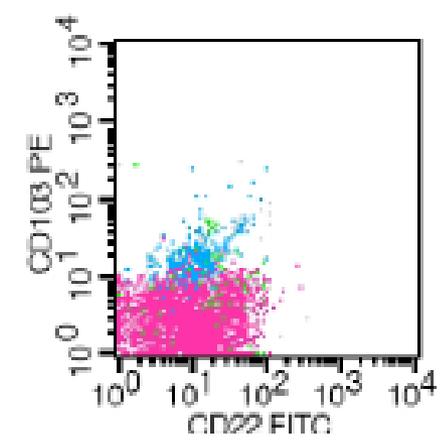
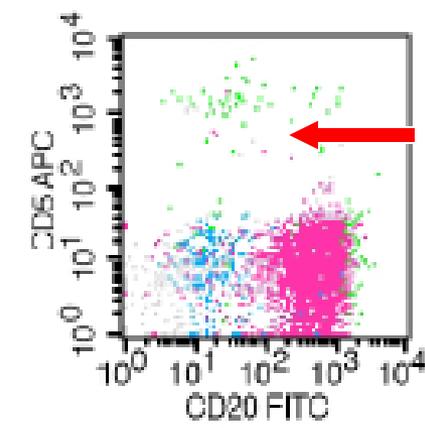
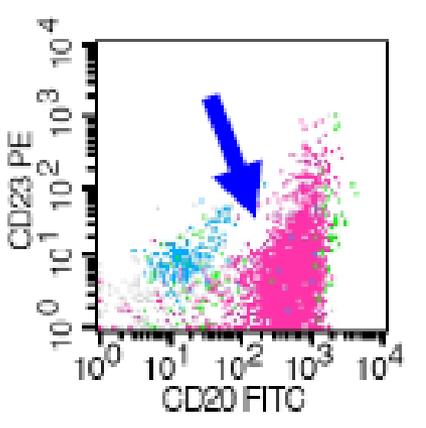
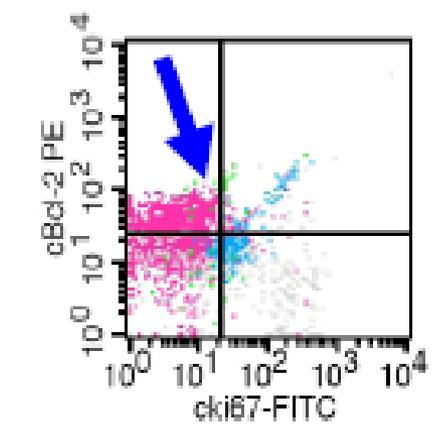
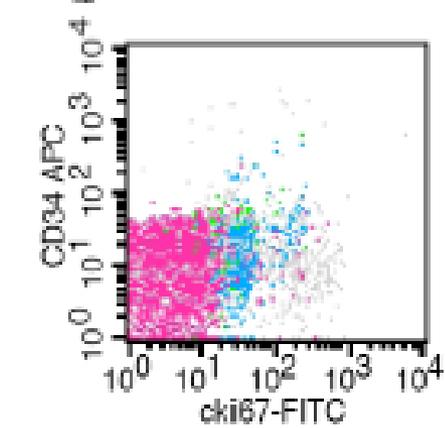
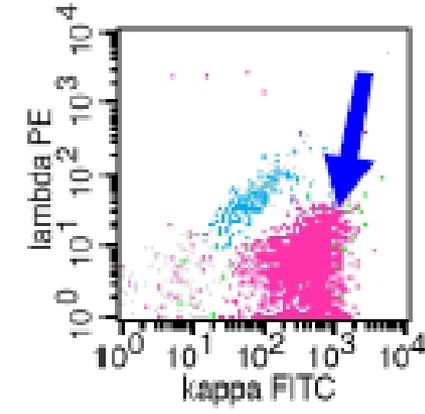
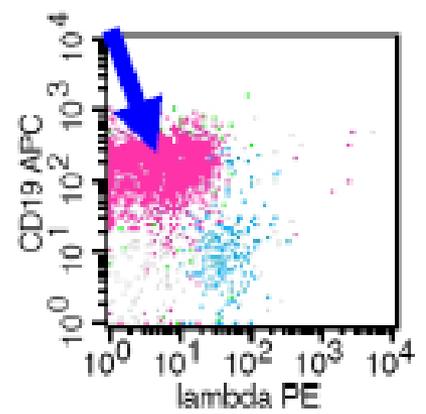


Gate: G1

Region	% Gated
R1	100.00
R2	1.93
R3	65.23
R4	6.48



FL-BM



➤ HCL

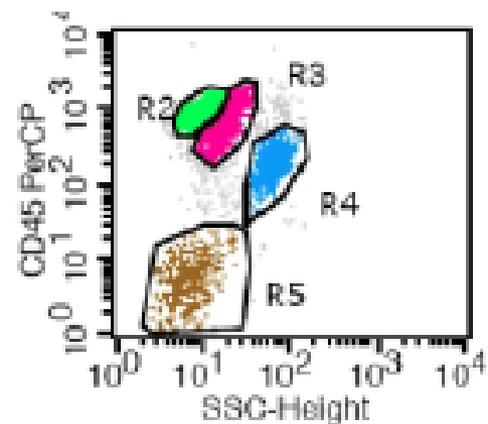
- 临床表现:

以脾肿大、全血细胞少为主要表现；外周血、骨髓或肝脾中可见“毛细胞”。白细胞数很少超过 $10 \times 10^9/L$ ，单核细胞减少为特征性表现

- 抗酒石酸酸性磷酸酶颗粒状强阳性(TRAP)

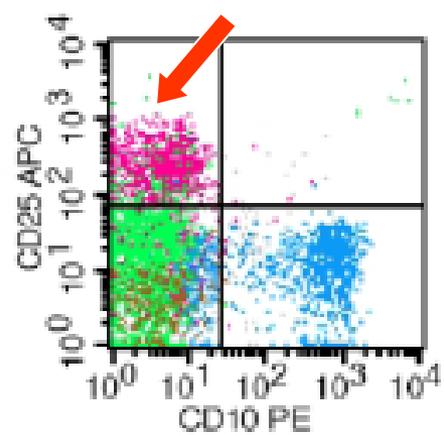
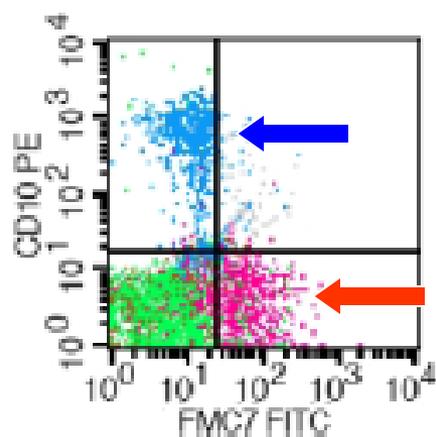
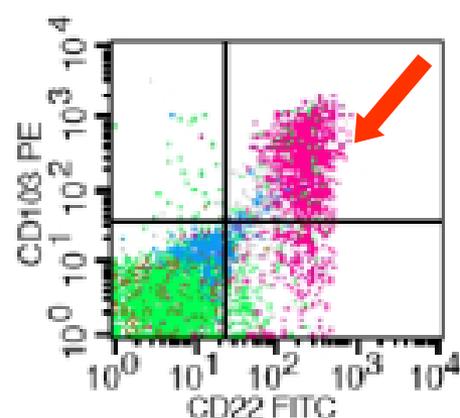
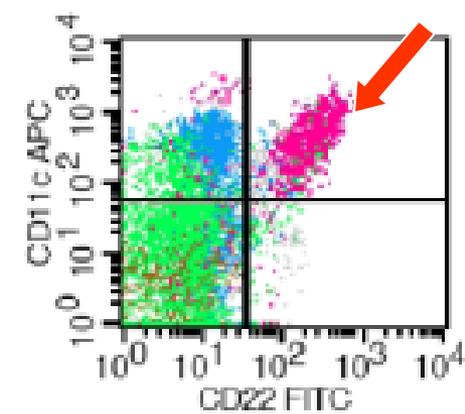
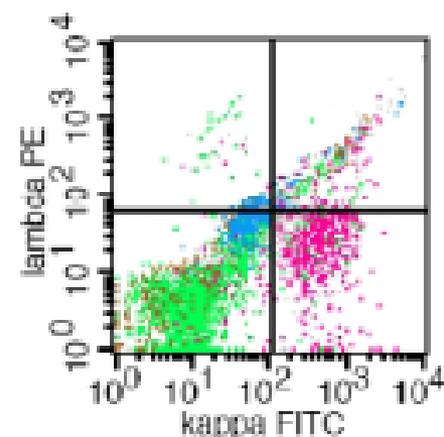
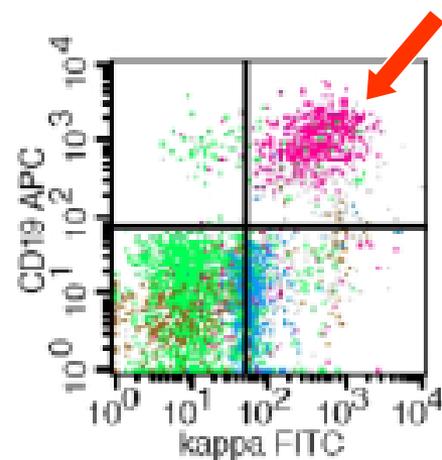
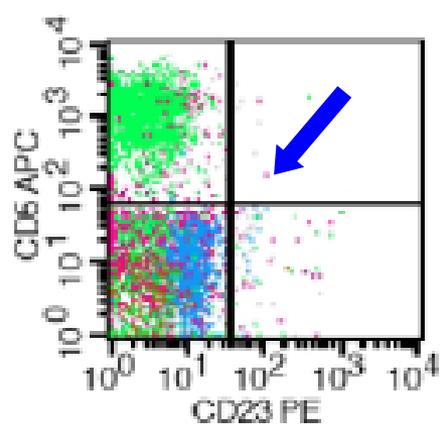
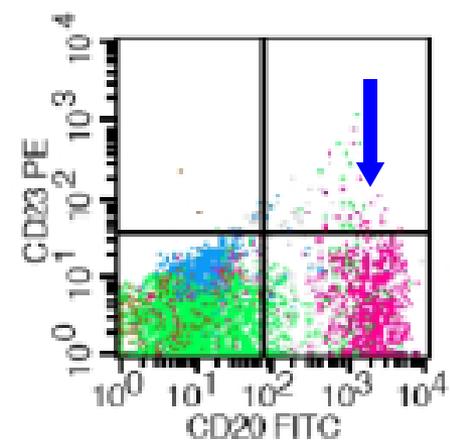
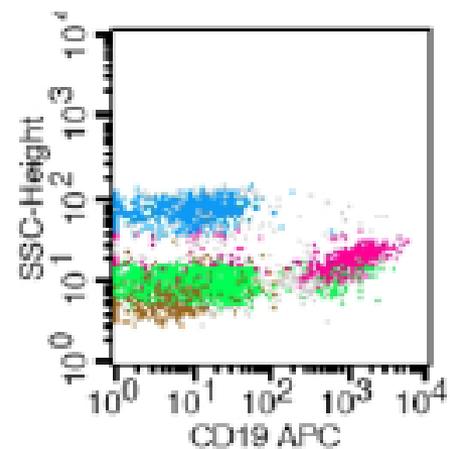
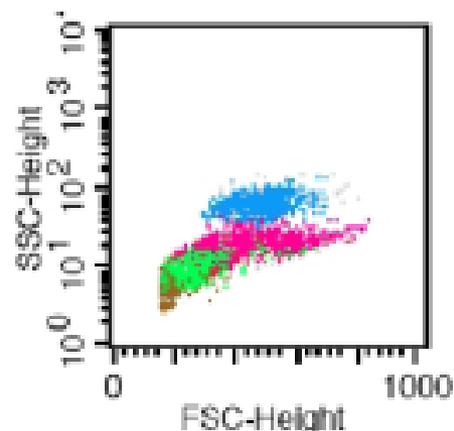
•HCL免疫表型

- ◆ FSC、SSC偏大
- ◆ 强表达B细胞标志CD20、CD19、CD22
- ◆ 表达单克隆性膜轻链
- ◆ 表达FMC7、CD103、CD11C、CD25、CD123、Annexin A1
- ◆ 少数病例可以表达CD10



Gate: G1

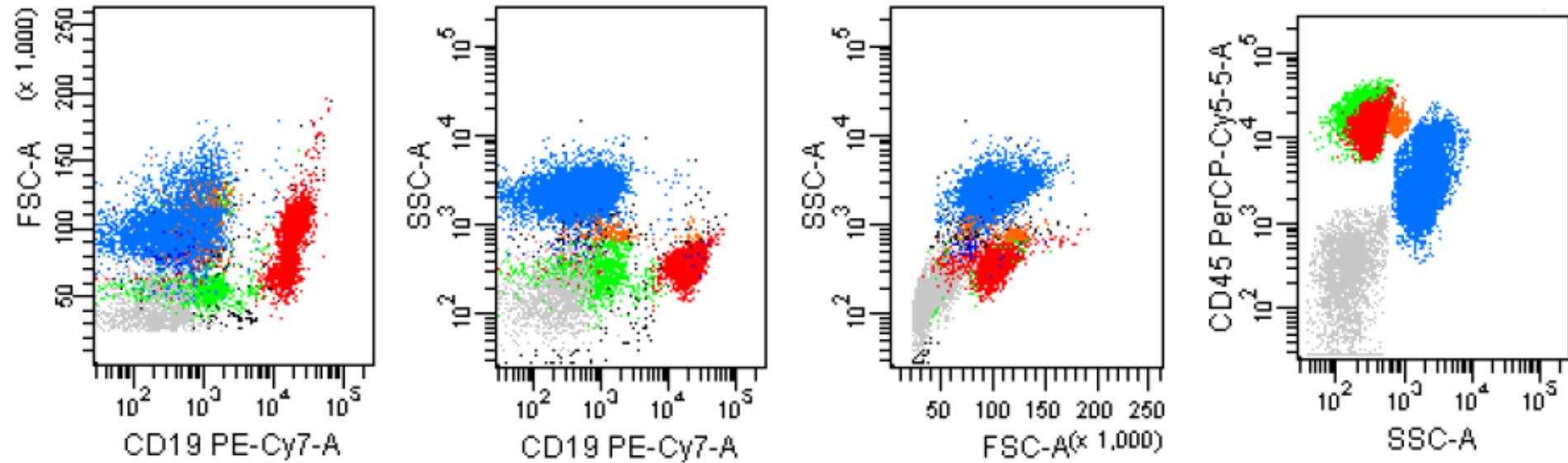
Region	% Gated
R1	100.00
R2	45.23
R3	21.47
R4	18.96
R5	7.35

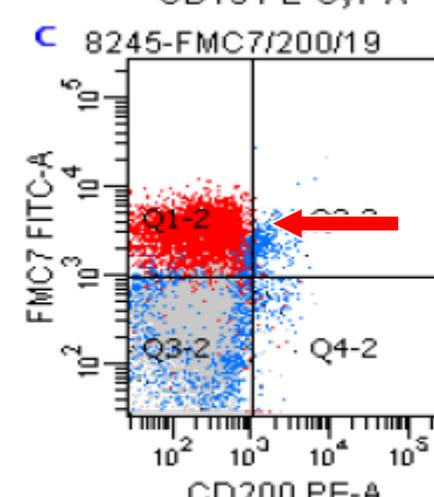
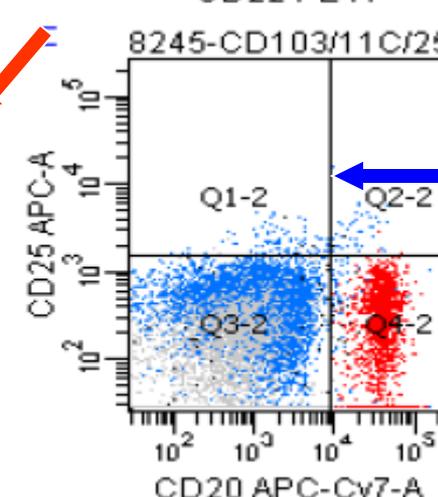
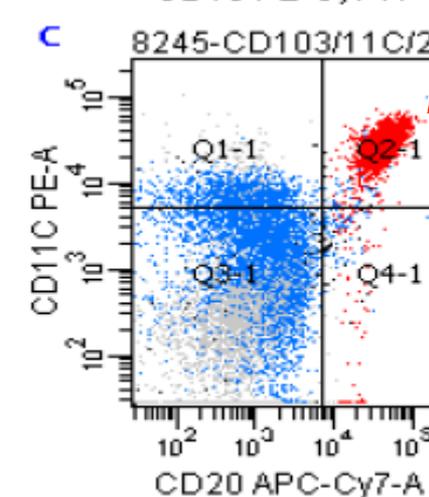
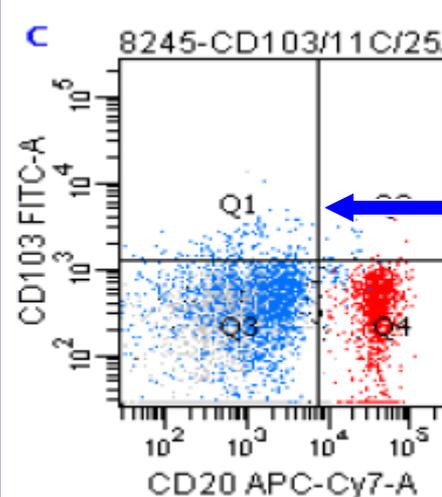
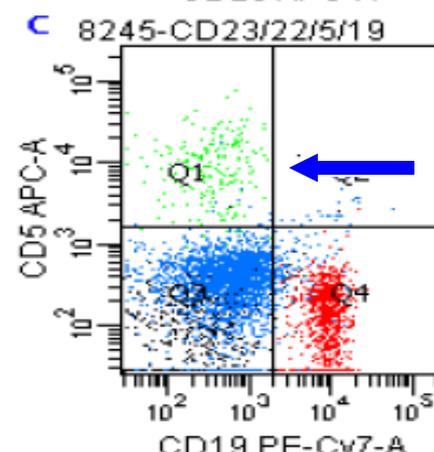
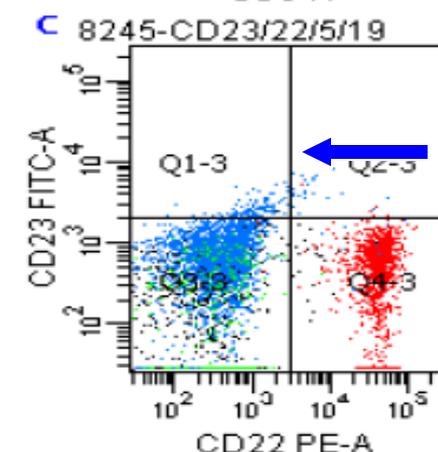
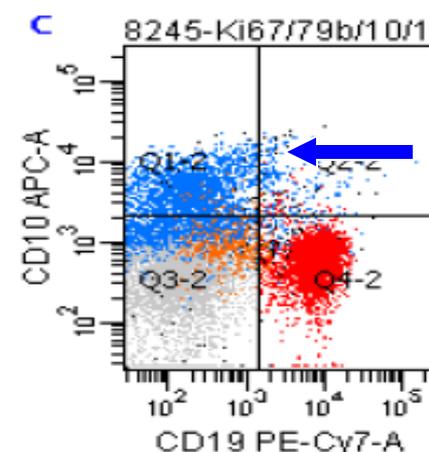
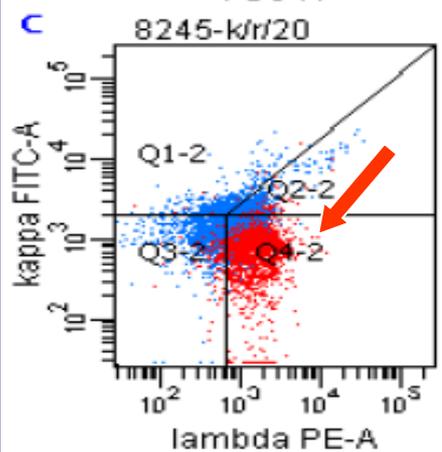
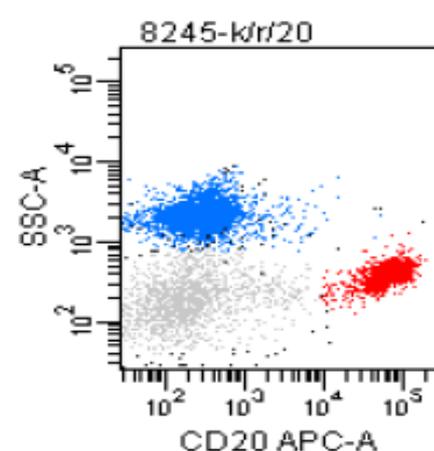
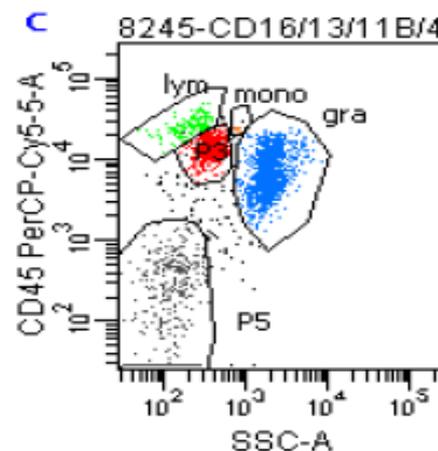
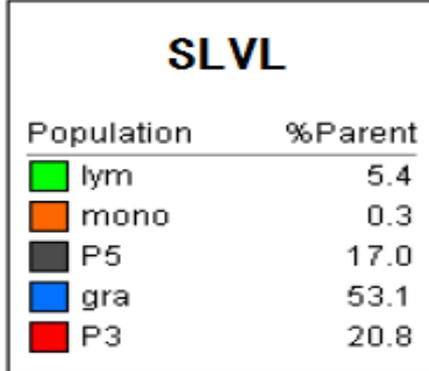
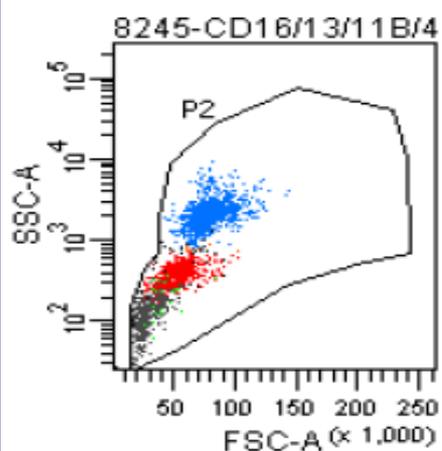


➤ CD5-CD10-CD103-

- PLL?
- SMZL?SLVL?
- WM?

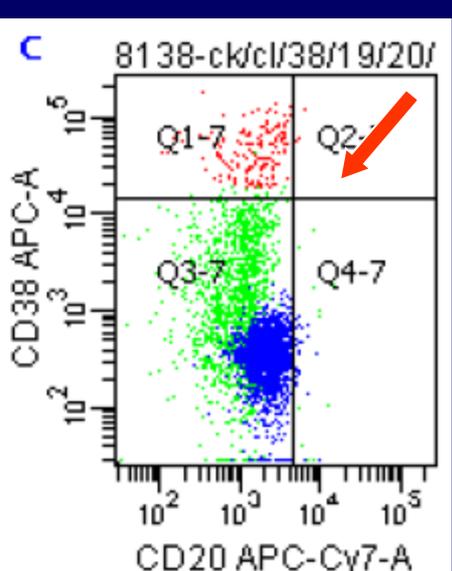
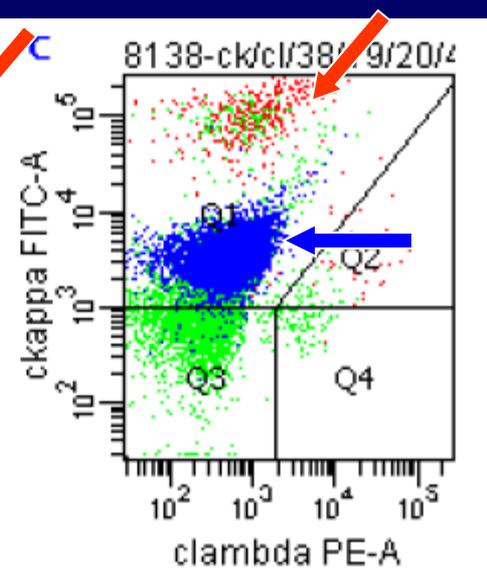
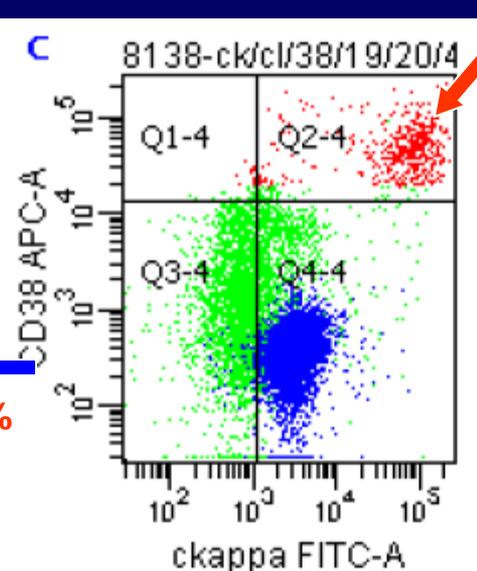
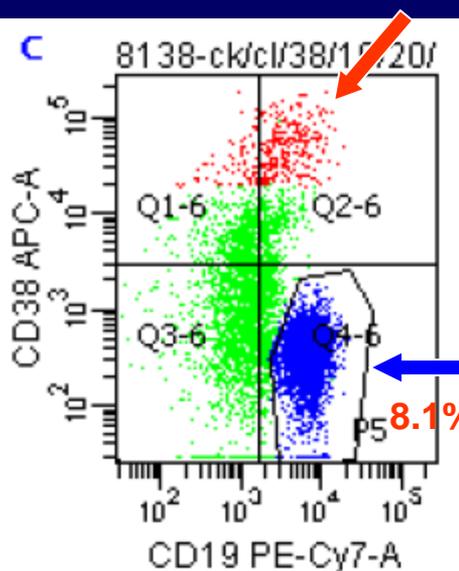
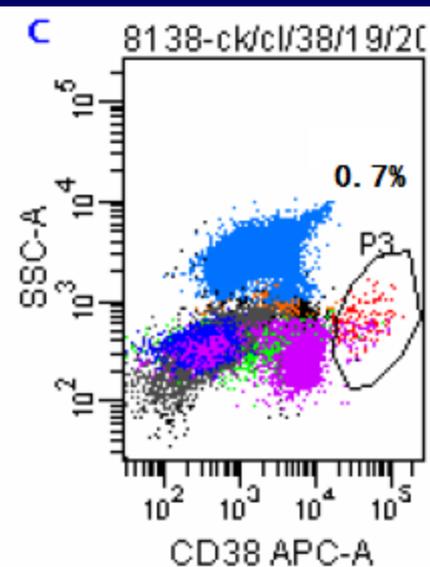
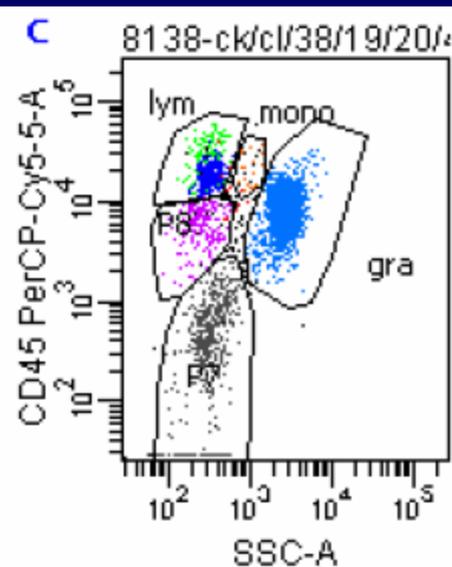
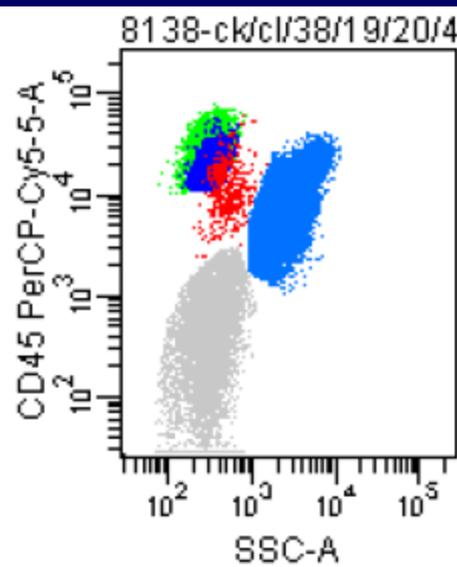
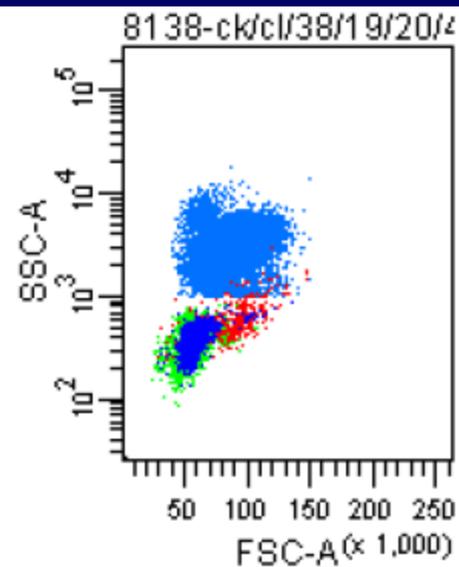
•SLVL



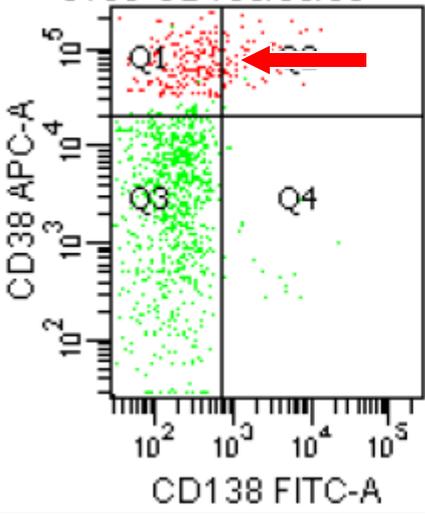


- 淋巴浆细胞淋巴瘤(LPL)/
华氏巨球蛋白血症(WM)

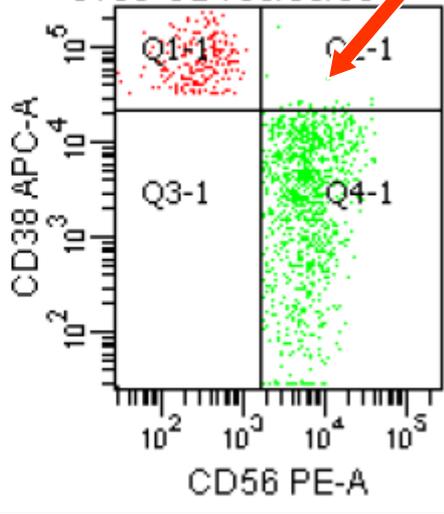
- ◆ 形态：可见淋巴浆样淋巴细胞
- ◆ WM者血清中可检测到单克隆Ig
- ◆ 典型的免疫表型：sIg和cIg+ (usually IgM)、CD23-、CD38+、



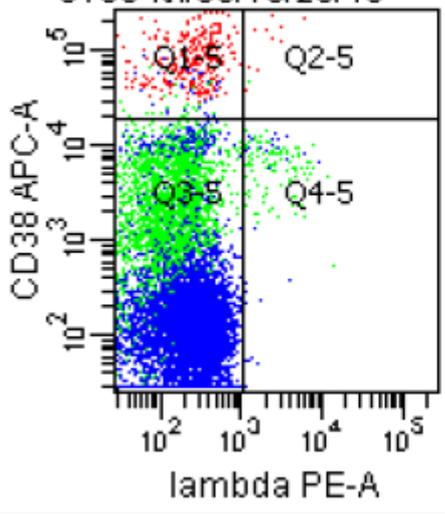
8138-CD138/56/38



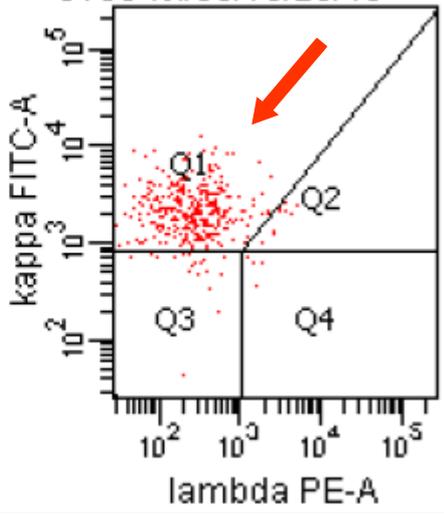
8138-CD138/56/38



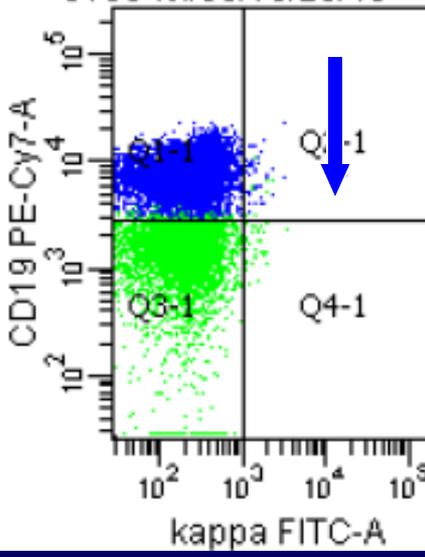
8138-kI/38/19/20/45



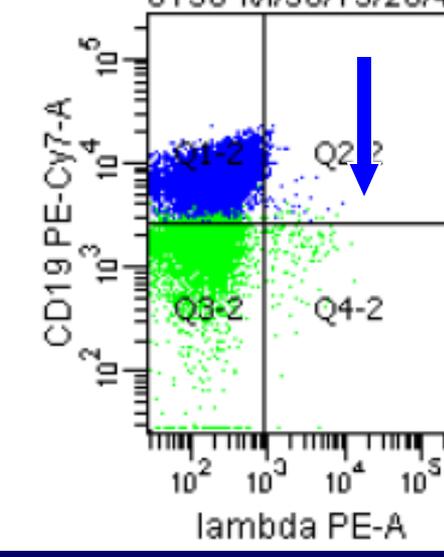
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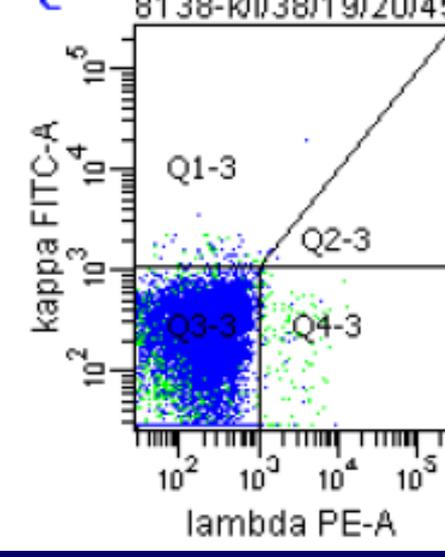
8138-kI/38/19/20/45



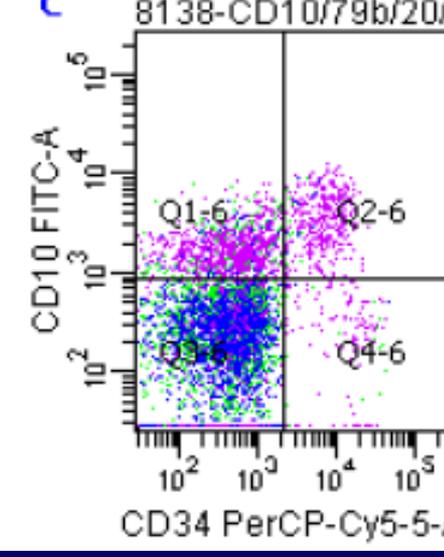
8138-kI/38/19/20/45



8138-kI/38/19/20/45

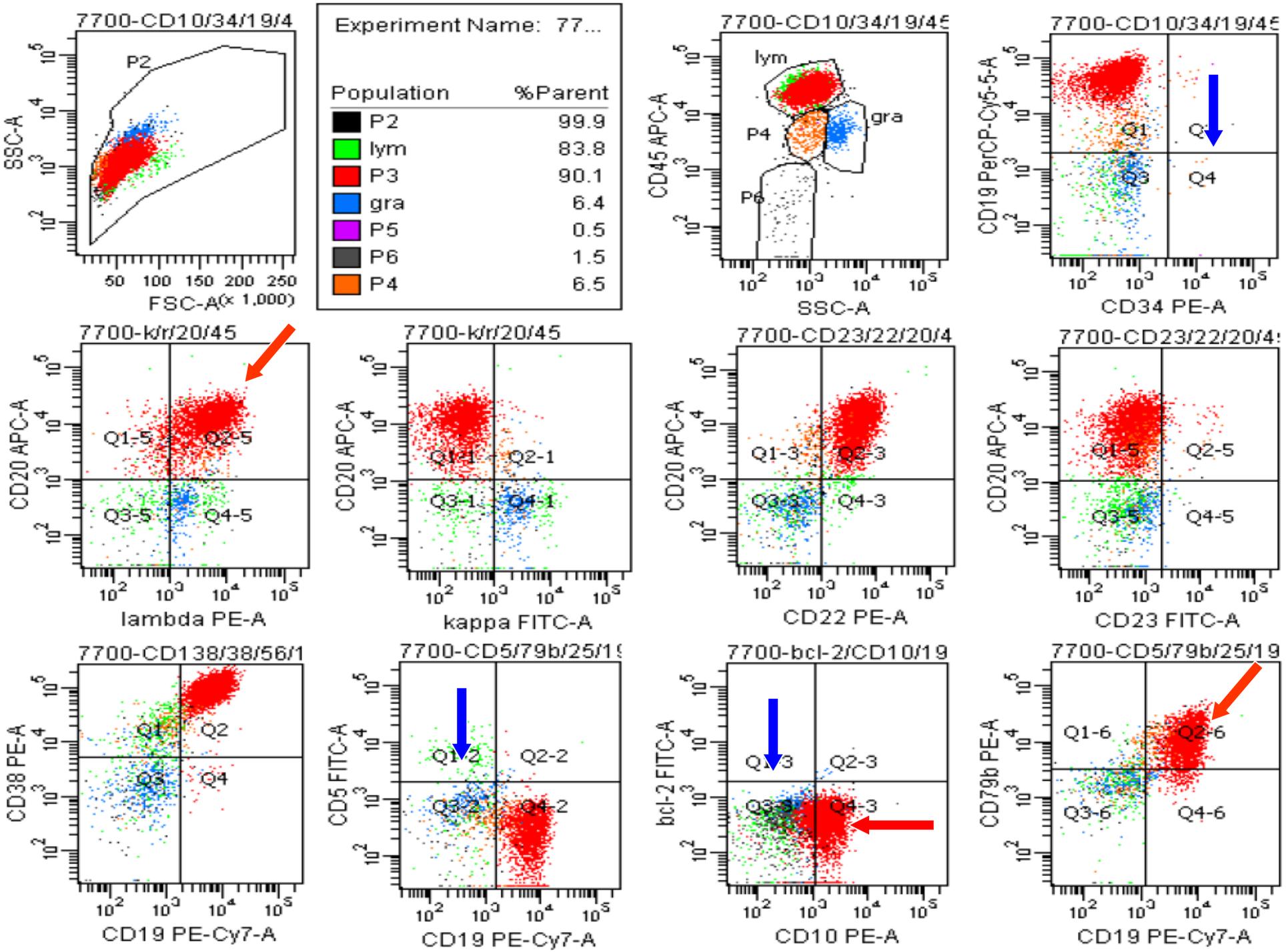


8138-CD10/79b/20/



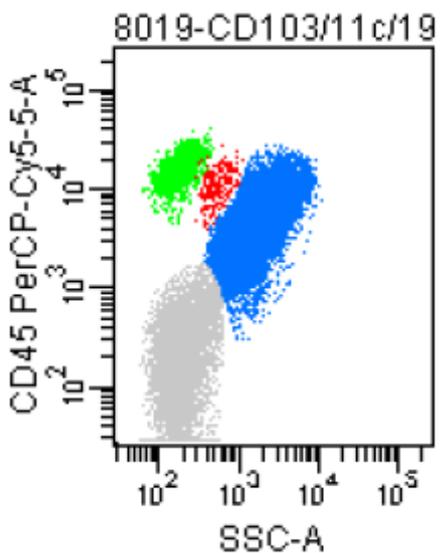
➤ Burkitt淋巴瘤

- CD10+: 90%
- CD20+: 100%
- Ki 67+: >90%
- Bcl-2+: 11%
- FSC介于淋巴和粒细胞之间



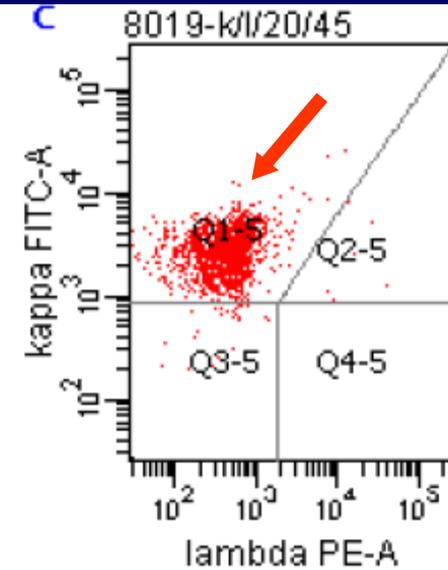
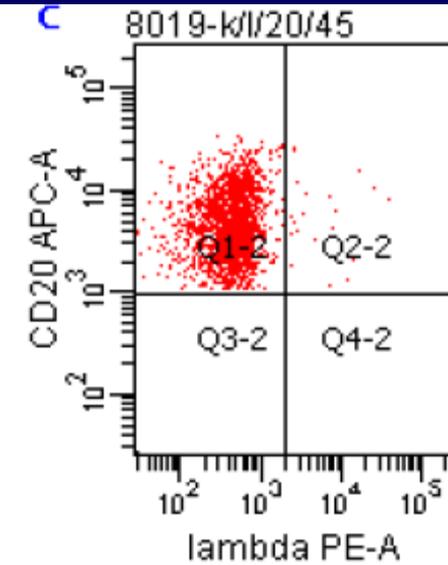
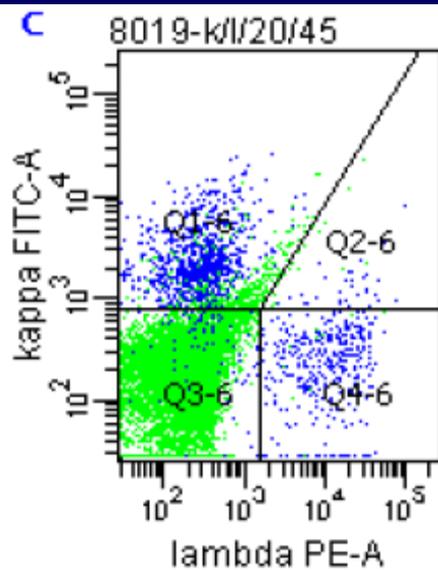
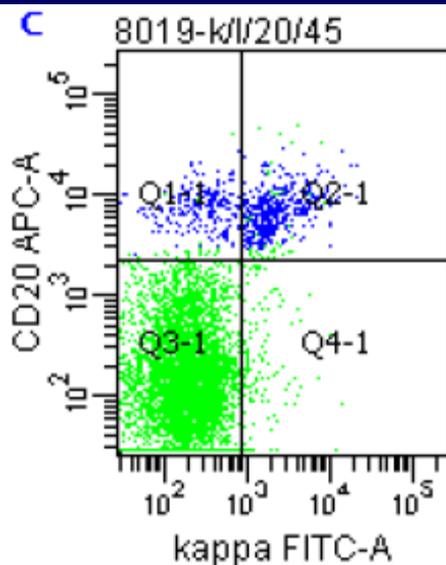
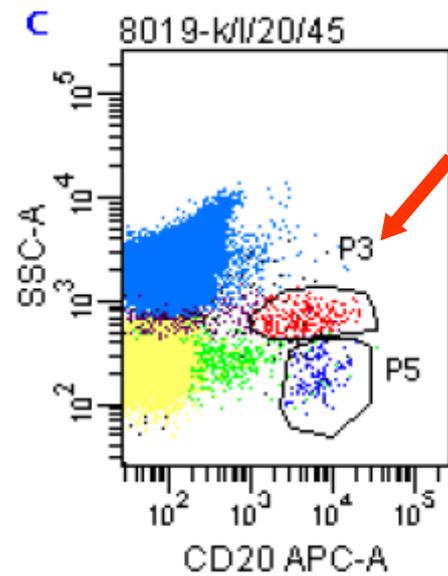
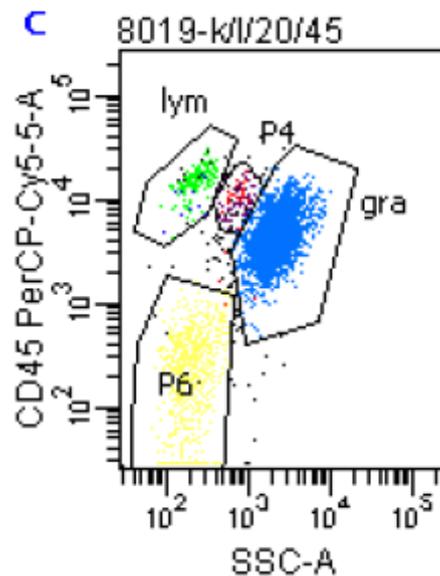
➤ DLBCL

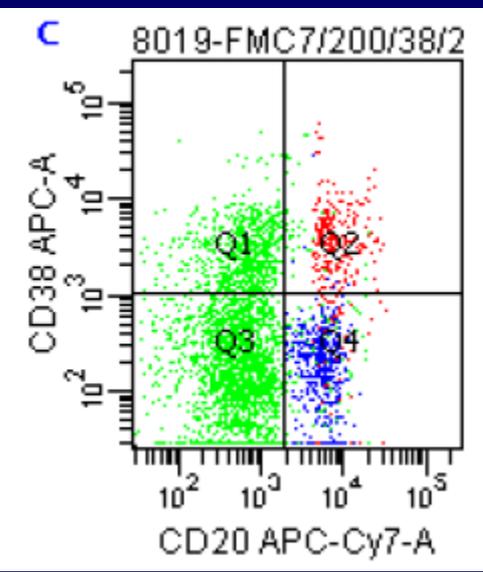
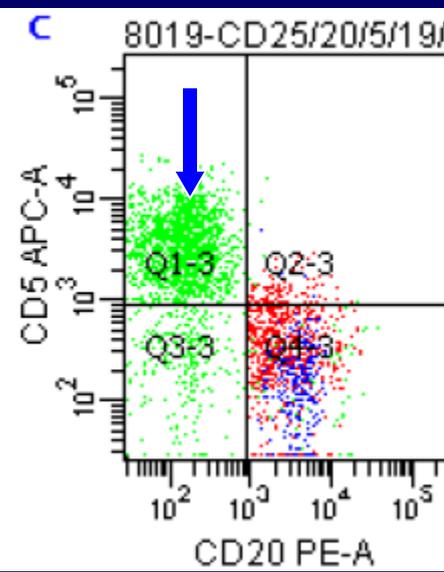
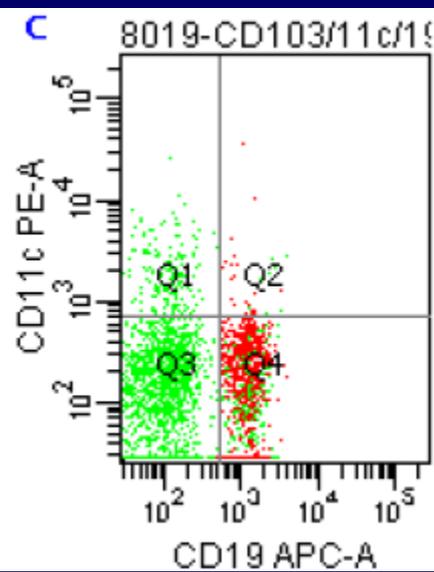
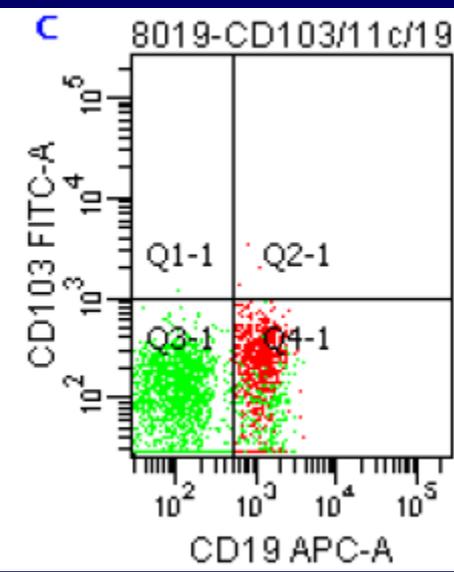
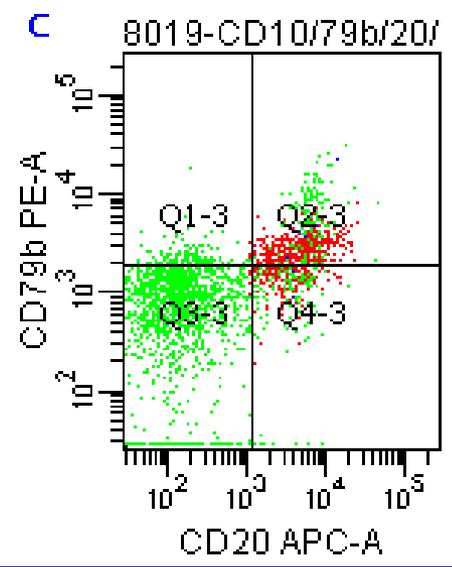
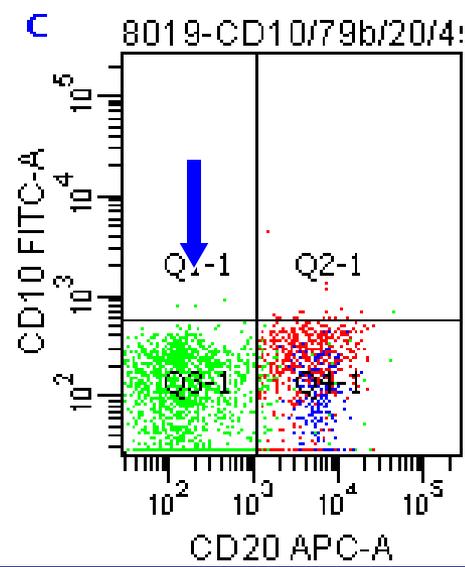
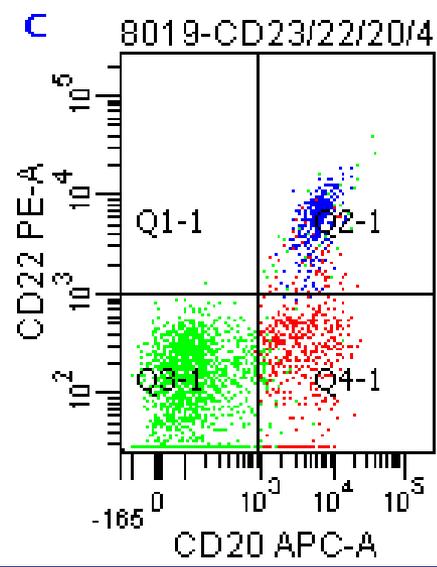
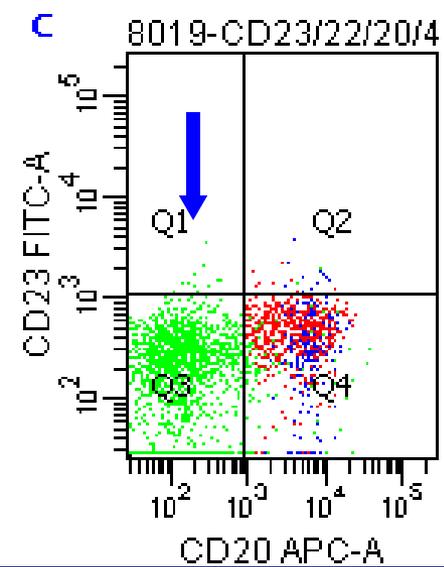
- CD10+: 40%
- CD20+: 100%
- Ki 67+: 30% ~ 70%
- Bcl-2+: 75%
- FSC大于粒细胞



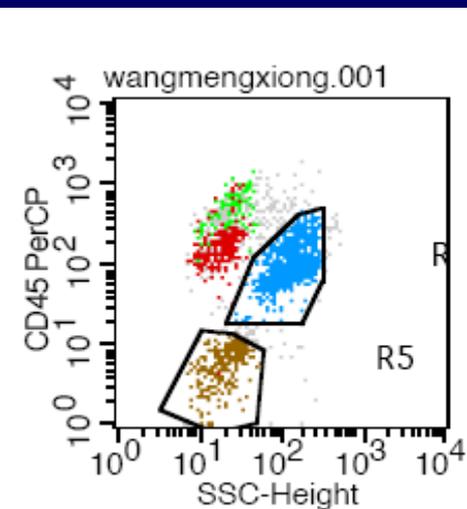
Experiment Name: 80...

Population	%Parent
P2	99.13
lym	2.63
P5	10.37
P6	16.08
P4	1.65
gra	78.16
P3	0.40





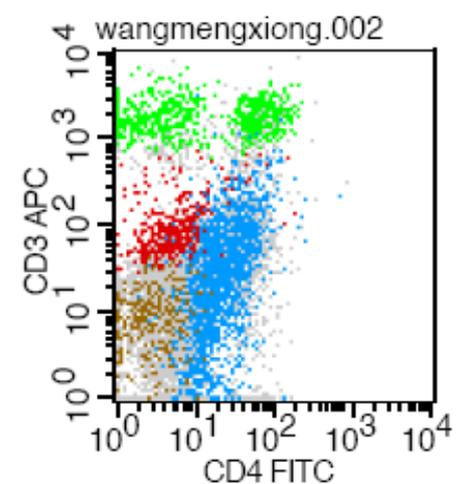
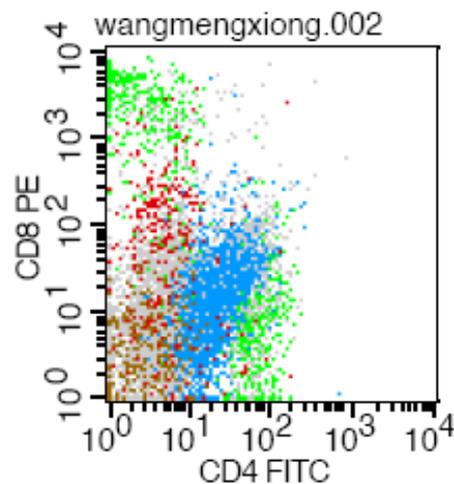
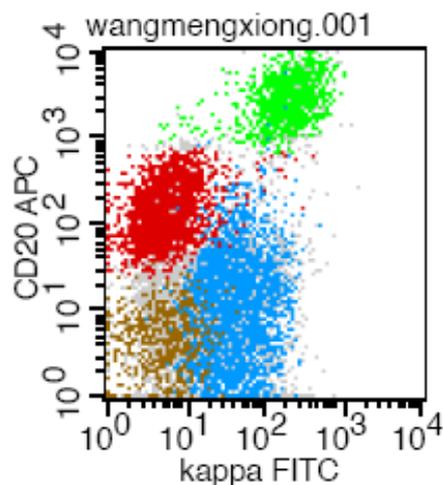
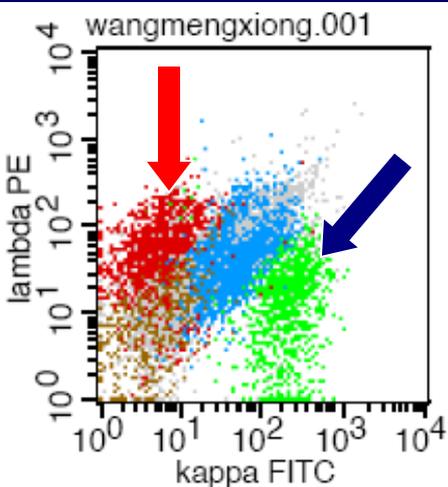
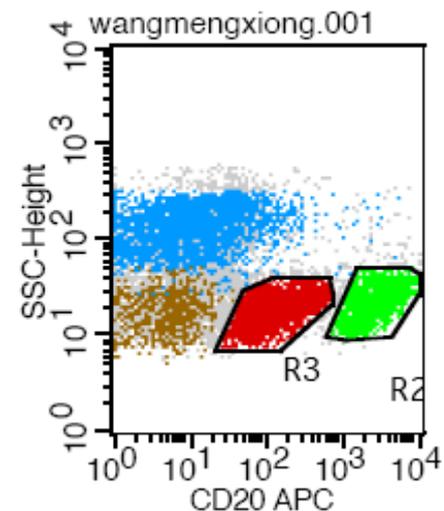
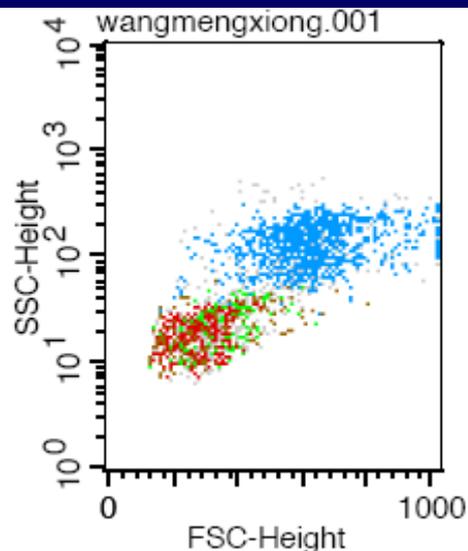
复合肿瘤



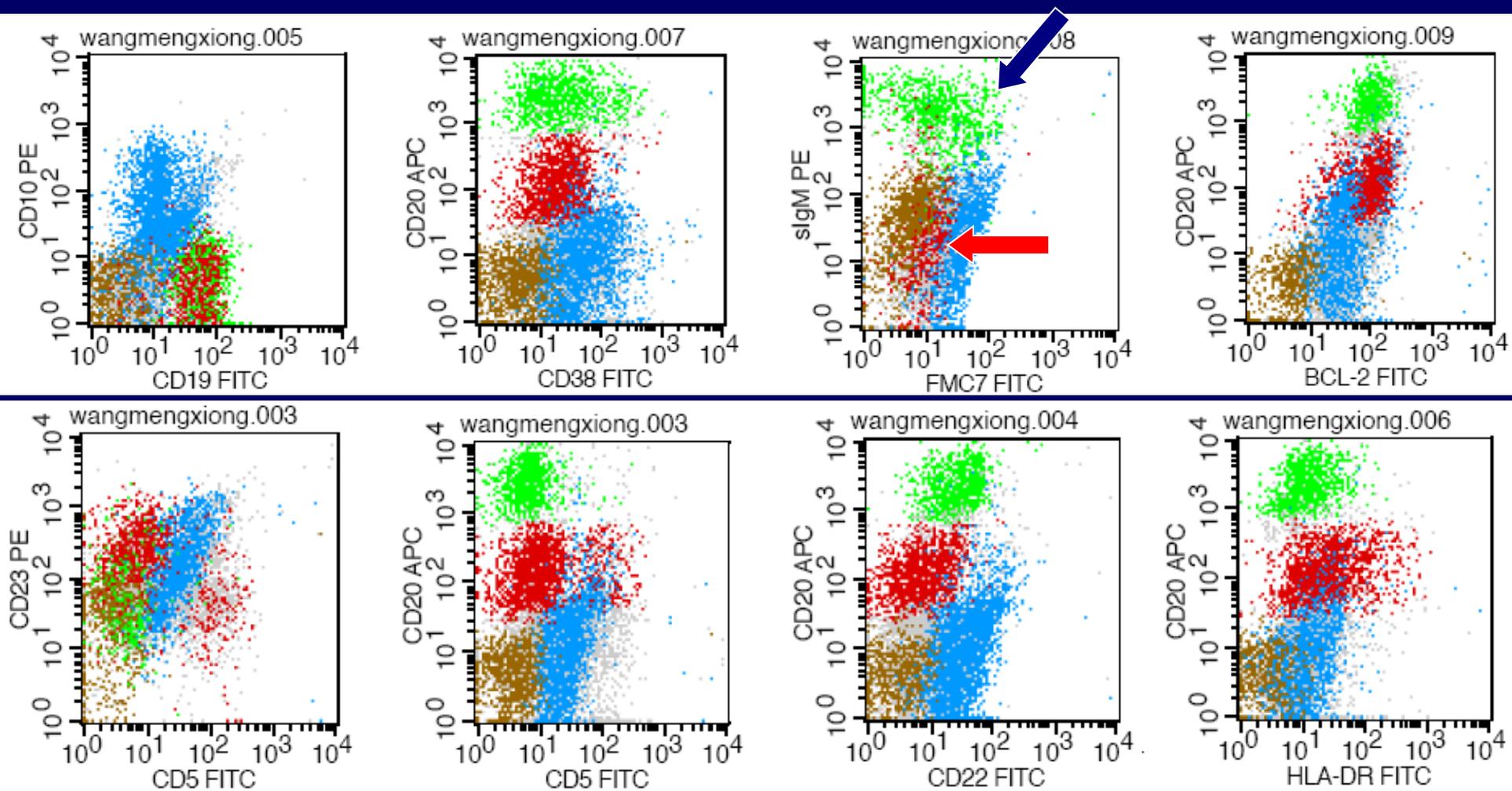
File: wangmengxiong.001

Gate: G1

Gate	% Gated
G1	100.00
G2	7.66
G3	15.63
G4	46.62
G5	12.56



检验结果：kappa链， IgG型



➤ B-NHL最低要求

- 基础标志： CD5、 CD10、 CD19、 CD20、
CD23、 CD79b或CD22 、 CD103、
kappa、 lambda、 ki 67等
- CD10+时加做TdT和CD34
- 选做标志： FMC7、 CD25、 CD11C、 bcl-2、
CD38、 CD200、 CD138、 IgM、 CD180、
CD81、 ckappa、 clambda、 ZAP-70

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Thank for your attention