Lentiviral vectors show dramatically increased efficiency of transduction of human leukemic cell lines

Moloney murine leukemia virus (MMLV)-based vectors have been widely used in vitro and in clinical studies. Subsequently, HIV-1 derived lentivectors were introduced[1] in which the env, vif, vpr, vpu and nef genes are deleted.[2] Another version of the lentivector has been developed which contains a 118 bp non-coding sequence from the HIV-1 pol gene.[3,4] We compare the capacity of these different retroviruses[5-7] to transduce human hematopoietic lines.

The T-cell lines CEM and JURKAT, the B-cell lines CAPO (EBV-LCL), BJAB, HBL1, HBL2, LAMC3, RAMOS, NC71 (Burkitt's lymphoma lines), IM9 (myeloma), DHL-4, KARPAS 422, and WSU-NHL (follicular lymphoma lines), the erythromyeloid cell line K562, the myeloid cell lines U937, HL60, THP1 and GFD8 were cultured in RPMI-1640.

The amphotrophic Phoenix cell line[5,8] and the PINCO plasmid were used as detailed previously.[6] For lentiviruses, 293T cells were transfected with 3.5 μg of envelope plasmid pMD.G, 6.5 μg packaging construct pCMVdR8.74, and 10 μg of transfer construct pRRLsin.hPGK.EGFP.Wpre (for the no cPPT virus)[9,10] or pRRLsin18.cPPT.hPGK.EGFP.Wpre (for the cPPT virus)[4] were co-precipitated in 1 mL. The following day the medium was changed and subsequently left for an additional 24 hours and finally filtered in the presence of Polybrene.

Next, 1 μL of viral supernatant was added to 0.5-1×10^6 cells in 24-well plates which were then spun at 1,800 rpm for 45 min at room temperature. After 6 hours of culture this procedure was repeated. Infected cells were analyzed for EGFP fluorescence by FACS after 5-8 days.

The viral titers were evaluated by infecting a known number of CEM cells with different volumes of retroviral supernatant as described previously[6] and are expressed as CEM transducing units (TU) per milliliter.

Cells were analyzed using a FacsCALIBUR instrument.

In a first series of experiments we compared a large panel of hematopoietic cell lines for efficiency of transduction by either a classical oncovector (PINCO) or a lentiviral vector (no cPPT), in both cases monitoring the green fluorescence conferred by the EGFP gene.

In order to compare the different efficiency of transduction and expression of the distinct viral preparations, we always used multiplicity of infections (MOI) well above 10 CEM TU per cell. In all cases the titers we obtained with all viruses were quite comparable, ranging in different preparations from 2×10^7 to 8×10^7 TU mL and we infected 0.5-1.0×10^6 cells with 1 mL of viral supernatant. Under these conditions we reached the maximal transduction efficiency for each virus as shown by titration experiments (data not shown).

As shown in Table 1, the data obtained with PINCO confirmed and extended our previous observations that good efficiency of transduction is obtained for most T, B and erythromyeloid cells lines (reaching up to 67 % with DHL-4 cells), whereas most myeloid cell lines showed very little if any susceptibility to transduction (1% for HL60 and GFD8, 7% for U937, somewhat higher for THP1).

We also tested the no cPPT lentiviral vector in identical experimental conditions. By contrast, the efficiency of transduction obtained with the no cPPT vector was much higher in all cases, reaching more than 90 % transduction in 6 out of 11 cell lines. Even in the case of myeloid cells, the percentage of GFP positive cells ranged from 43 to 99 %.

Finally we compared the efficiency of infection using both the PPT and no PPT vectors. The results obtained on a large panel of B leukemia and lymphoma cell lines are shown in Table 2. In all cases the two viruses showed very high percentages of transduction, with a small but reproducible increase in the percent and in the MFI of the positive cells by the cPPT relative to the no cPPT virus.

Here we have used two different transfer vectors, one derived from the pRRL.SIN-18 and containing the EGFP gene under the control of the PGK promoter[9] (here called no cPPT) and the other which differs only for the presence of a 118 bp sequence from the pol gene of HIV-1 (here called cPPT).[4] Both lentivectors have been pseudotyped with VSV-G envelop protein. Furthermore their capacity to infect cell lines was compared to that of the Moloney derived oncoviral vector PINCO as released by the amphotrophic Phoenix cell line.[5,6]

We demonstrate that both lentiviruses show a very high effi-

Table 1. Efficiency of transduction of different hematopoietic cell lines.

	PINCO[a]		no cPPT[b]	
Cell line	*% transduction*	*MFI*	*% transduction*	*MFI (s.d.)*
T cells				
CEM	34	342	76 (8)	174 (50)
JURKAT	40	395	96 (3)	1176 (276)
B cells				
CAPO	9	1171	46 (4)	211 (31)
BJAB	60	817	96 (3)	390 (39)
IM9	26	1514	69 (22)	290 (203)
DHL4	67	1253	94 (1)	314 (138)
Myeloid				
U937	7	421	98 (1)	441 (176)
HL60	1	150	53 (14)	131 (7)
THP1	31	516	99 (1)	1633 (131)
GFD8	1	69	43 (8)	105 (18)
Erythromyeloid				
K562	38	447	97 (4)	557 (343)

The indicated cell lines were infected with either PINCO or no cPPT virus.
[a]The results shown are the % positive cells and MFI, as measured by FACS analysis of a representative experiment.
[b]The results shown are the mean % positive cells and mean MFI, as measured by FACS analysis of several experiments and standard deviation in brackets (s.d.).

Table 2. Efficiency of transduction of different B cell lines.

	no cPPT		cPPT	
Cell line	*% transduction*	*MFI*	*% transduction*	*MFI*
MC71	62	361	67	439
KARPAS 422	62	158	61	229
DHL4	93	250	92	443
WSU-NHL	27	181	38	329
HBL1	71	674	74	847
HBL2	37	199	40	297
LAMC3	51	253	61	333
RAMOS	28	144	40	192
CAPO	48	23	60	33

The indicated cell lines were infected with either the cPPT or no cPPT virus.
The results shown are the % positive cells and MFI, as measured by FACS analysis.

ciency of transduction in a wide variety of hematopoietic cell lineages including T lymphoid, B lymphoid, erythromyeloid and myeloid cells with a slightly superior efficiency of the cPPT version with respect to the no cPPT. Due to our experimental conditons the dramatically higher efficiency of transduction shown by the two lentivectors as compared to the more classical oncovector Pinco should not be attributed to differences in the titers obtained or in the infection procedures, but rather can be attributed to differences in receptor usage or in reverse transcription and integration steps. Overall, these data must be considered in the perspective of possible future clinical developments, in particular the possibility of transferring genes in leukemic cells in order to produce cell vaccines or even to manipulate the transforming genetic program directly. Finally, evolution of the lentiviral vectors in three separate plasmid systems and the introduction of suicide LTRs will probably meet clinically acceptable standards in the near future.

Federica Bambacioni,° Chiara Casati,° Marta Serafini,§° Massimiliano Manganini,§* Josée Golay,§ Martino Introna§

°Centro "M. Tettamanti", Clinica Pediatrica, Università di Milano Bicocca, Ospedale San Gerardo, Monza; §Istituto di Ricerche Farmacologiche "Mario Negri", Milan; *Divisione di Ematologia, Ospedali Riuniti di Bergamo, Italy

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Correspondence: Dr. Martino Introna, MD, Laboratory of Molecular Immunohematology, Istituto di Ricerche Farmacologiche "Mario Negri", via Eritrea 62, 20157 Milan, Italy. Phone: international+39.02.39014547.
Fax: international +39.02.33200231.
E-mail: martino@marionegri.it

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